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Composition and potential utilization of crop residues and forages within the digestive tract of ruminants as predicted by laboratory techniques

by

Geraldo Maria da Cruz

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ABBREVIATIONS AND NOMENCLATURE

ADF	acid detergent fiber
ALF	alfalfa hay
A.O.A.C.	Association of Official Analytical Chemists
App. Abs Ash	apparent absorption of ash
" Ca	" calcium
" Cu	" copper
" Mg	" magnesium
" Mn	" manganese
" P	" phosphorus
" Zn	" zinc
App. DCP	apparent digestibility of crude protein
" DDM	" dry matter
" DE	" energy
" DOM	" organic matter
ARC	Agricultural Research Council
ASA	acid soluble ash
ATP	adenosine triphosphate
C	temperature, degree centrigrade
Ca	calcium
CF	crude fiber
Cl	chlorine
cm	centimeter
Co	cobalt
CP	crude protein
C.P.	chemically pure
Cr	chromium
CSS	corn stover silage
cstovsil	corn stover silage
Cu	copper
d	day
DAPA	diaminopimelic acid
CV	coefficient of variation
CWC	cell wall constituents
DAsh	disappearance of ash
DCa	" calcium
DCP	" crude protein
DCu	" copper
DDM	" dry matter
DK	" potassium
DM	dry matter
DMD	<u>in vivo</u> dry matter digestibility
DMg	disappearance of magnesium
DMn	" manganese
DNA	deoxyribonucleic acid
DOM	disappearance of organic matter
DP	" phosphorus
DPW	dry poultry waste

DZn	disappearance of zinc
EDTA	ethylenediamine tetraacetate
EE	ether extract
F	fluorine
Fe	iron
g	gram
grnd	ground
h	hour
HCl	hydrochloric acid
I	iodine
IVDMD	*in vitro* dry matter disappearance
K	potassium
Kg,kg	kilogram
l	liter
La	lanthanum
mcg	microgram
mg	milligram
min	minute
ml	milliliter
mM	millimolar
mm	millimeter
Mn	manganese
Mo	molybdenum
N	nitrogen
Na	sodium
NAN	nonammonia nitrogen
NB	nitrogen balance
NDF	neutral detergent fiber
NFE	nitrogen-free extract
Ni	nickel
NRC	National Research Council
Nylon bag technique, *in situ* technique, synthetic fiber bag technique, dacron bag technique:	all these expressions have been used interchangeably in this dissertation; a technique used to measure the utilization of feedstuffs contained in a bag made of a specific synthetic fiber
OM	organic matter
P	phosphorus
PER	protein efficiency ratio
Potential availability, potential utilization:	release of any element from bag or test tube containing a feed sample upon digestion in the rumen and/or incubation with enzymes
RNA	ribonucleic acid
RSD	residual standard deviation
S	sulfur
s-c	sun-cured
Se	selenium

Si	silicon
Sn	tin
sol. CHO	soluble carbohydrates
solv-extd	solvent-extracted
spods	soybean pods
sstalks	soybean stalks
sstover	soybean stover
TABSCa	calculated true absorption of calcium
TABSCu	" copper
TABSMg	" magnesium
TABSP	" phosphorus
TABSZn	" zinc
TA Cu	true availability of copper
TDN	total digestible nutrients
Tr.	trial
USDA	United States Department of Agriculture
V	vanadium
w	with
wo	without
w/sol	with solubles
Wt	weight
$Wt.^{75}$	metabolic body weight
Zn	zinc

INTRODUCTION

Most of the areas of the world, to varying extents, have a low-quality roughage feed potential, whether it is a crop resource or residue of grain production. Approximately one-half of the total biomass produced by grain crops is vegetative material. It has been estimated that 225 million metric tons of straw, stubble and stalks are left on the ground as waste after grain harvest in the United States each year (Klopfenstein et al., 1979). Corn grain residues comprise approximately two-thirds of that amount (USDA, 1974). This represents an unconventional form of feed for ruminants. It could be especially important where weather conditions cause a forage deficient period due either to extreme cold or to a prolonged dry season.

Cell wall constituents (cellulose, hemicellulose, and lignin) comprise from 60 to 84% of most crop residues (Vetter, 1975), thus contributing a vast organic form of energy to ruminant animals. The complete diets for one-half the dairy production system, which includes the growing replacement heifer, the dry cow, and the average cow during the last 4 to 5 months of lactation, require only 60% total digestible nutrients (TDN) in their feed dry matter (DM). Crop residues may provide the main energy source of the diet of these animals, even though a chemical treatment may be needed in order for this value of 60% TDN to be attained (Klopfenstein and

Owen, 1981). If crop residues are to become a major feed source for Beef and Dairy animals, greater emphasis must be given to the composition and availability of nutrients in these roughages.

In general, there seems to be disagreement about an average value for the true availability of calcium in feedstuffs. While the National Research Council (NRC) committee assumed it to be 45%, the Agricultural Research Council (ARC) committee concluded that it was 68% (Miller, 1983).

The difficulties associated with mineral digestibility and mineral balance studies, such as contamination of samples, level of mineral intake, physiological status of the animal, have hampered the progress needed in this area. Also, the extent of solubilization or disappearance of minerals from roughages during their digestion in the alimentary tract of ruminants is a neglected subject (Field, 1981). There appears to be only one quantitative study (Playne et al., 1978b) showing that the disappearance of nitrogen and minerals from tropical hays occurred in two stages during their digestion.

The purpose of the present research was to determine the nitrogen and mineral composition of selected forages, crop residues and crop residue plant parts, as well as to evaluate the potential availability (utilization) of the dry matter, nitrogen and some useful minerals contained in these feedstuffs as potential feed supplies for ruminant farm species when measured by laboratory techniques.

LITERATURE REVIEW

Essential Mineral Elements in Ruminant Nutrition

Minerals make up only 4 to 6% of the body of a vertebrate animal, but because of their diverse roles in the body processes, they are important in the entire field of nutritional biochemistry (IMC, 1973). In addition to the seven major mineral elements--calcium (Ca), chlorine (Cl), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), and sulfur (S)--14 trace elements are probably required in the nutrition of higher animals (Miller and Neathery, 1977). These 14 trace elements are: chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), silicon (Si), tin (Sn), vanadium (V), and zinc (Zn). Of these, the evidence for essentiality has been developed only within the last two decades for six (Cr, Si, V, F, Sn, and Ni). According to Miller and Neathery (1977), the importance of these six elements in practical feeding and nutrition, if any, is largely unknown. The minor mineral ions which are believed to be required as supplemental elements for livestock and poultry are Co, Cu, I, Fe, Mn, Se, and Zn, according to Ammerman and Miller (1972).

The major building blocks of biology are covalent molecules, polymers, proteins, polysaccharides, RNA (ribonucleic acid) and DNA (deoxyribonucleic acid), and two types of phases

discrete from the aqueous phase--organic bilayer lipid membranes and salts. The bilayer lipid membranes are largely based on phospholipids; RNA and DNA are polymers based on phosphate ester monomers; protein synthesis process needs the energy of a phosphate condensate, ATP (adenosine triphosphate); and one of the salts in bone is calcium phosphate (Williams, 1979).

Mineral Deficiencies and Their Determination

It is generally accepted that P is the mineral element most commonly found to be deficient in forages grazed by livestock throughout the world (Hall, 1976). Malnutrition on pastures of subnormal mineral content is due directly to the failure of the diet to supply the necessary inorganic materials for constructional purposes and for maintaining the normal balance of mineral elements in the blood and tissues (Woodman and Evans, 1930).

Brum et al. (1980) found deficient levels of P, Ca and Mg in certain soils in the state of Mato Grosso in Brazil, and deficient levels of P and Ca in the pastures if they were to supply the total needs of these mineral elements to a lactating Beef cow. Mineral elements in forage are often direct reflections of levels in the soils (Hall, 1976), plant maturity (Gomide et al., 1969; Schwartz and Kafkafi, 1978) and season (Robinson and Sageman, 1967).

The criteria used by Heinemann et al. (1957) to judge the

adequacy of P in alfalfa hay for rabbits were: growth of the animal, mature body weight, breeding efficiency, ash-Ca and P in bones, breaking strength of humerus, and inorganic serum P. They concluded that alfalfa hay produced on a soil of low available P was not adequate for normal rabbit nutrition. Since a positive soil-plant-animal interrelationship has been demonstrated, the level of mineral elements in soils along with the soil physical and chemical properties could be used as criteria to detect mineral deficiencies in animals.

When working with grazing animals, it is difficult to get representative pasture samples and those samples collected from esophageal fistulated animals are contaminated with salivary secretions. In order to solve this problem, Little et al. (1977) labelled salivary P with ^{32}P. Thus, the P content of consumed feed could be calculated from the degree of reduction in salivary specific activity by the feed P.

A simple technique to show deficiencies of mineral elements in animals would be to determine the content of inorganic mineral elements in plasma (Brum et al., 1980; Cohen, 1973a,b; Heinemann et al., 1957). However, the correlation coefficients between pasture P content and the concentration of inorganic P in plasma was not significant (Cohen, 1973a). Furthermore, the levels of inorganic P and Ca in plasma were not altered by P supplementation (Cohen, 1973b). Forty-five percent of the lactating Beef cows analyzed for plasma inor-

ganic P would not be considered deficient in P while grazing a P deficient pasture in Brazil (Brum et al., 1980). In those works, neither the level of mineral elements in plasma, nor the level of mineral elements in hair could be used as criteria to detect deficiencies of mineral elements in cattle.

Another technique used to measure the mineral status of bovines is the rib-bone biopsy. Brum et al. (1980) found that 100, 100, and 85% of the lactating Beef cows analyzed for rib-bone P, Mg and Ca, respectively, were deficient in those mineral elements when grazing pastures grown on soils deficient in those mineral elements even though the herbage was not deficient in Mg. The relationship between the P content of pasture (X) and that in dry fat-free rib-bone (Y) was given by equation $Y = 7.5 + 33.3\ X$ (Cohen, 1973a). Moreover, the supplementation of P increased the amount of P in the dry fat-free rib-bone (Cohen, 1973b). The amount of Ca in bone (Y) was influenced by pasture Ca content (X_1) and bone P content (X_2) (Cohen, 1973b). This relationship was described by the equation $Y = 51.0 + 140.8\ X_1 - 3.33\ X_2$. The measurement of bone P and Ca provides the most sensitive indication of the P and Ca status of Beef cattle. Besides, when the supplementation of macro-mineral elements has no effect on liveweight, its effect on the mineralization of bone tissue may justify its use (Cohen, 1973b).

The low feed efficiency, reduced serum alkaline phospha-

tase and low blood hemoglobin observed in Holstein calves were due not only to the indirect influence of reduced feed intake but also to the Zn deficiency per se (Miller et al., 1965). Radioactive Zn (^{65}Zn) uptake by cells of whole blood in vitro did not differentiate dietary Zn deficiencies from other factors which may reduce plasma Zn under "field conditions" (Chesters and Will, 1978).

Requirements for Crude Protein (CP), Macro- and Micro-Mineral Elements by Beef and Dairy Cattle

Lebdosoekojo et al. (1980) investigating the mineral nutrition of Beef cattle in the Savanna Grasslands area of the Eastern plains of Colombia found that the content of P and Na and, in some instances, Ca and Cu in herbage was insufficient for optimum beef production. Brum et al. (1980) found that the grasses in some regions of Mato Grosso in Brazil were insufficient to supply the needs of Ca, Mg, and P of lactating Beef cows. Robinson and Sageman (1967) came to the same conclusion studying the nutritive value of some pasture species in Northwestern Australia. There were marked changes in the quality of grasses between the wet and dry seasons in this latter work. This effect was particularly striking with CP and P content which fell to low levels during the late dry season (.01 to .04% P).

Kubota et al. (1980) studying the regional patterns of the incidence of grass tetany found that it seemed more closely

associated with grasses having less than .2% Mg than with grasses having K/Ca+Mg ratios of 2.2 or more.

More specific requirements for CP, macro- and micro-mineral elements by beef and dairy cattle than the figures cited above are presented in Table 1. The only disagreement among the values for CP presented in this table is between the NRC (1976) for Beef Cattle and the NRC (1978) for Dairy Cattle for nonlactating cows. The Ca requirements listed in the ARC (1980) are generally higher than the NRC (1976) for Beef Cattle, except for the lactating cow where they give similar values. On the contrary, the NRC (1978) for Dairy Cattle and the ARC (1980) give similar values for the growing animals and nonlactating cows but not for the lactating cow, where the NRC (1978) gives much higher values. The NRC (1978) for Dairy Cattle shows higher P requirements for the lactating cow than either the ARC (1980) or NRC (1976) for Beef Cattle; however, the NRC (1978) lists similar P requirements for the growing and nonlactating animals as compared to the ARC (1980) values.

According to Miller (1983), the reason for the higher mineral requirements listed by the NRC (1978) is because it was designed to provide minimum concentrations of mineral elements needed for maximum performance under a variety of practical conditions. Thus, the NRC (1978) (Nutrient Requirements of Dairy Cattle) includes safety margins which the NRC (1976) and ARC (1980) generally do not have.

Table 1. Requirement for CP, TDN, Ca, P, Mg, Zn, Mn, and Cu for growing animals, lactating and nonlactating cows[a]

	CP	TDN	Ca	P	Mg	Zn	Mn	Cu	Reference
	----------	----------	%-	----------		-------	ppm-	-------	
Growth									
300-kg calf									
Maintenance	8.6	-	.23	.15	.17	-	-	-	ARC (1980)
	8.6	55	.18	.18	.04	-	-	-	NRC (1976)
1 kg/day of gain	10.0	-	.43	.23	.13	22-35	20-25	9-15	ARC (1980)
	10.4	79	.33	.29	.10	20-30	1-10	4	NRC (1976)
	11.0	67	.40	.26	.16	40	40	10	NRC (1978)
Nonlactating cow	-	-	.32	.26	.17	13-21	20-25	13-20	ARC (1980)
	5.9	52	.18	.18	-	-	20	-	NRC (1976)
	11.0	60	.37	.26	.16	40	40	10	NRC (1978)
Lactating cow	13-14	-	.34	.31	.19	19-27	20-25	8-11	ARC (1980)
	9.2	52	.36	.34	.18	-	-	-	NRC (1976)
	13-16	63-75	.60	.40	.20	40	40	10	NRC (1978)

[a]Concentration of the element in the total diet dry matter.

The average availability of Ca and P in all feedstuffs shown in Table 2 was used by the NRC (1978), ARC (1980), and NRC (1976) committees to estimate the Ca and P requirements of ruminant livestock.

Table 2. Average availability of Ca and P in all feedstuffs used for estimating the requirements for these two mineral elements

	NRC (1978)	ARC (1980)	NRC (1976)
	----------%	----------	
Ca	45	68	70
P	55	58	70

The low P level for nonlactating beef cows suggested by the NRC (1976) is in agreement with the work of Bass et al. (1981) which suggested that a total intake of only about 10-12 g P per day was adequate to maintain normal blood P concentration, voluntary straw intake and digestibility of diet by the beef cows over the last 16 weeks of pregnancy.

The needs for Ca and P for lactation estimated from Ca and P balances with vitamin D supplemented cows were 5.0 g Ca and 2.3 g P per kilogram (kg) of milk (Ward et al., 1972). These values are even higher than the figures given by the NRC (1978) for Dairy Cattle, which are the highest values cited in Table 1. Yoon and Evans (1981) suggested that the NRC

(1978) recommendations for diet Ca should be increased by 30 to 40% for the high producing dairy cows. Kincaid et al. (1981) reported that maximum milk yields and performance could not be obtained with .3% dietary phosphorus as suggested by ARC (1980).

The ARC (1980) and the NRC (1978) for Dairy Cattle give similar requirements for Mg for all classes of animals compared in this table; however, the NRC (1976) for Beef Cattle lists smaller requirements for Mg for the growing animal.

The NRC (1978) for Dairy Cattle gives higher requirements for Zn and Mn and similar Cu requirements when compared to those values of the ARC (1980).

Macromineral Requirements for the Growth of Rumen Microorganisms

Burroughs et al. (1951) presented evidence that other mineral elements besides Na, K, Ca, Mg, Cl, S, and P were involved in rumen bacterial physiology. These mineral elements listed above were routinely added to the artificial saliva used in the *in vitro* rumen fermentation technique.

The range of concentrations of mineral elements, in micrograms (mcg)/ml of fermentation medium, found to support maximum *in vitro* cellulose digestion were: P 40-80 (Anderson et al., 1956); S 10-500; Mg 20-160; Ca 50-300 (Hubbert et al., 1958a); K 100-400 (Hubbert et al., 1958b).

Bryant et al. (1959) reported on the minimal requirements

of a rumen cellulolytic bacterium (Bacterioids succinogenus). About 60 mcg PO_4^{-3}, 40-60 mcg NH_3, 2 mcg Mg^{+2} and 10 mcg of Ca^{+2} per ml of medium were required for maximum growth.

Effect of Fertilization on the Level of Crude Protein and Mineral Elements in Pastures and Crops

Nitrogen (N) fertilization generally causes an increase in yield (Plucknett and Fox, 1965) and in CP content of grasses (Rosero et al., 1980; Glenn et al., 1980).

The ratio of total N to total S, which ranged from 3.2:1 to 15.6:1 in perennial ryegrass, generally increased with increasing rates of N and K fertilization (Whitehead et al., 1978). Glenn et al. (1980) also found that with N fertilization alone the ratio of total N to total S increased on the average from 6.9:1 to 17.2:1 in orchardgrass and tall fescue. With only S fertilization, the ratio of total N to total S decreased only from 6.1:1 to 5.3:1 while with both N and S fertilization, the ratio of total N to total S increased from 6.9:1 to 11.8:1.

There was no change in the content of mineral elements (Ca, Mg, P, K, Cu) in several tropical forages with N fertilization (Gomide et al., 1969), and on the P content of a legume forage (Desmodium intortum) in the associated grass (pangola) legume plots (Plucknett and Fox, 1965). However, the fertilization with N caused a decrease in P content in pangola grass (Plucknett and Fox, 1965) and a decrease in Ca and P in orchardgrass and Kenhy tall fescue (Rosero et al.,

1980). An increase in the content of Mn in tropical forages (Gomide et al., 1969) and Zn in Coastal Bermudagrass (Miller et al., 1964) was observed with N fertilization.

A positive linear relationship between either Ca or Mg uptake and the amount of N uptake was observed in wheat plants but not in corn plants with N fertilization (Schwartz and Kafkafi, 1978).

Phosphorus fertilization alone caused an increase in the P content of pangola grass with no increase in yield. When both N and P were applied, the highest yields were obtained with modest P fertilization and with a small decline in the P content of the herbage. Potassium fertilization caused a drastic reduction in the Mg uptake in corn plants (Schwartz and Kafkafi, 1978). Copper sulfate application to Cu-deficient soils raised the Cu concentration in the herbage only slightly, with species and/or varieties differing little in their response to applied Cu (Forbes and Gelman, 1981).

Effect of Mineral and Protein Supplementation

On milk production

Dairy cows fed diets low in P (.3% P) had lower milk yields than cows fed diets containing .54% P (Kincaid et al., 1981). Fishwick et al. (1977) reported a depression in milk yields of beef cows as measured by a reduced calf live-weight gain when the cows were fed 12 g P/day as compared to a di-

calcium phosphate supplemented diet containing 28 g P/day. The reduced milk production in the latter work may have been due to a reduced roughage (straw) intake in this group.

Milk production efficiency per unit of ingested diet DM increased from 1.74, 2.25, 2.29 to 2.55 with increments of diet Ca from .38, .55, .72 to .89% in the diet (Yoon and Evans, 1981).

On liveweight change

Phosphorus supplementation (35 or 70 g/week) had no effect on liveweight gain of Beef cattle grazing a CP and P deficient carpet grass (Cohen, 1972). However, Playne (1969) reported that dicalcium phosphate supplementation promoted an increase in the liveweight of sheep fed stylosanthes but not spear grass.

When the CP level of the total diet (straw-based) increased from 5.2 to 6.2%, cows gained more weight and increased feed consumption by 17%. Cows also gained more weight when the low protein (5.2% CP) diet was supplemented with Mg than those that were fed a similar diet without Mg (Mathison et al., 1981).

Supplementation with either iodized salt or a complete mineral mixture to Beef cattle promoted similar weight gains when the animals were grazing pastures in the savanna region in Brazil (Graca, 1979).

Ivan and Grieve (1975) reported that the supplementation

of a barley-urea basal ration with trace elements did not affect body weight gain of Holstein calves. However, Zn deficiency caused a reduced weight gain in Holstein calves, an effect which was not reversible when the animals were transferred to a sufficient-Zn diet (Miller et al., 1965).

On feed intake and utilization of nutrients

Some researchers conducting long-term experiments with P deficient diets have demonstrated that a reduced cow feed intake may occur after a long mineral depletion period and the stress of parturition and lactation (Fishwick et al., 1977; Bass et al., 1981; Kincaid et al., 1981). Playne (1969) demonstrated an increase in DM intake by sheep when it was supplemented with dicalcium phosphate; however, the phosphate supplementation had no consistent effect on the intake of spear grass.

According to Woodman and Evans (1930), the failure of stock to thrive on mineral-deficient pastures cannot be explained by assuming that the low mineral content is responsible for the lack of palatability in the herbage and a consequent depression of appetite in the grazing animals. Neither is there any evidence that a shortage of minerals causes the herbage to be digested any less efficiently than normal cultivated herbage of similar maturity and organic composition.

Supplementation with P did not change the digestibility of DM in sheep (Playne, 1969; Weir et al., 1958) and in buffalo (Agarwala and Nath, 1980). Also, no change was observed in

the digestibility of DM, N and energy in steers (Cohen, 1972). Furthermore, supplementation with trace elements did not affect digestibility coefficients of DM, N and energy in Holstein calves (Ivan and Grieve, 1975). The DM digestibility in rats was not affected by Zn intake. Neither was the digestibility of DM affected by Zn deficiency in calves and goats (Miller et al., 1966).

On the contrary, rations supplemented with dolomitic limestone were digested significantly less than ratios containing either magnesium oxide or magnesium carbonate (Moore et al., 1971). Thus, some factor other than magnesium carbonate in dolomitic limestone caused the depression in carbohydrate digestibility, and seemed to be related to the rate of passage. Both Fishwick et al. (1977) and Bass et al. (1981) have shown a reduced intake and digestibility of organic matter (OM) when beef cows were fed low P straw-based diets for long periods during pregnancy and early lactation. These effects were seen after the stress of parturition and beginning of lactation.

Although most of the investigators have demonstrated that supplementation with mineral elements did not improve the digestibility of DM, it did increase the efficiency of milk production (Kincaid et al., 1981; Yoon and Evans, 1981). The reduced feed efficiency in Zn-deficient animals reported by Miller et al. (1965) was caused by a reduced utilization of digested nutrients (Miller et al., 1966). Weigand and Kirch-

gessner (1977) reported that inadequate Zn intake impaired the utilization of dietary protein for growth in rats as indicated by the protein efficiency ratio (PER).

Absorption and Excretion of Mineral Elements

Calcium

Perry et al. (1967) showed a net secretion of Ca in the upper small intestine of calves. They also showed that absorption of this element was greatest during the passage of digesta through the small intestine and few changes occurred in the intestinal concentration of Ca in the passage through the cecum and large intestine.

There is now clear evidence that both sheep and cattle absorb Ca from their gut according to need and that they can alter the efficiency of absorption to meet a change in requirement (Scott and McLean, 1981). Abdel-Hafeez et al. (1982) reported that when the diet of sheep was changed from normal to a low Ca one, there was an increase in the efficiency of net Ca absorption from a Thiry-Vella loop of jejunum. This theory was further confirmed by intravenous infusion of a Ca chelating agent (EDTA, ethylenediamine tetraacetate) which increased the efficiency of net Ca absorption from the jejunum loop. This effect was demonstrated by the use of a radioisotope, ^{47}Ca, which showed an increase in the true absorption rate of Ca. Braithwaite (1978a) also demonstrated that the absorption

of Ca is regulated according to need. He infused Ca directly into the bloodstream of Ca-deficient wethers, following their receiving an adequate Ca intake. The rate of Ca retention increased only slightly during infusion. The surplus Ca was compensated for by a decrease in the rate of absorption and increase in the rate of urinary Ca excretion. According to the author, these findings support the theory that, at maximum retention, the rate of Ca absorption becomes regulated by the rate at which Ca can be stored in bone.

The net Ca absorption rate from a Thiry-Vella loop of jejunum of sheep was increased by the addition of 1-OH cholecalciferol (1-OH-D_3) to the loop fluid (Abdel-Hafeez et al., 1982). Braithwaite (1978b) also noted that the rate of Ca absorption was substantially higher in the 1-OH-D_3 treated ewes than in controls. Although the rate of bone accretion increased slightly, the increase in skeletal content of Ca resulted mainly from a decrease in the rate of bone resorption.

The decrease in Ca absorption observed when Ca was infused directly into the bloodstream of Ca-deficient wethers, already receiving an adequate Ca intake, was attributed to a decrease in the rate of active absorption. The continuing low rate of absorption was attributed to diffusion (Braithwaite, 1978a). By the use of Thiry-Vella loop of jejunum, a two-component relationship between net absorption rate of Ca and intraluminal Ca concentration was demonstrated by Fox et al. (1978) in

conscious pigs. An initial rapid absorption rate from 0 to 4 mM (millimolar) was found, then a lower linear rate was observed from approximately 5 to the highest (25 mM) Ca concentration tested.

Jones and Luthman (1978) studying a feeding-induced hypocalcemia demonstrated that .4 kg/day of concentrate was necessary to induce the drop in serum Ca in sheep and 3 kg concentrate per day in heifers. Isotope studies showed that the fall in serum Ca was probably not caused by a reduced gastrointestinal Ca uptake.

Lomba et al. (1978) showed correlations between Ca absorption and the daily ingested amounts of the alkaline ions, Na and K, minus the sum of stable acid ions, chloride, sulfate and phosphate. The excess of the anions over the cations, in rations maintaining a positive Ca balance but not in rations allowing a negative Ca balance, increases the absorption of Ca from the intestine. Phosphorus not alone, but with chloride and sulfate, is the most important component of this effect.

The form (organic vs inorganic) of dietary P had no significant effect on absorption and retention of Ca (Dutton and Fontenot, 1967), but dietary P deficiency in sheep reduced the efficiency of intestinal Ca absorption. This effect was associated with a reduction in plasma level of 1,25 $(OH)_2D_3$) (Abdel-Hafeez et al., 1982).

The results of experiments with pregnant ewes of Braithwaite (1978a) suggested that the decreased Ca-retention observed in protein-deficient animals was a result of decreased rate of Ca absorption. In fact, Howe and Beecher (1981) reported that increasing dietary protein resulted in significantly decreased fecal Ca output accompanied by an increase in the Ca content of urine in rats fed either .35% or .80% P in the diet.

Destruction of oxalate by ruminal bacteria has been demonstrated. Campos et al. (1980) showed that the addition of sodium oxalate to the diet of goats did not reduce Ca absorption; however, when it was introduced directly into the duodenum, Ca absorption decreased drastically. This effect was also seen studying Ca absorption in jejunal loops. The absorption of Ca from calcium oxalate in goats was considerably lower than that from calcium chloride (Campos et al., 1980). Calcium-containing crystals in alfalfa were described by Ward et al. (1979), using scanning electron microscopy and energy-dispersive X-ray techniques. Most intact crystals appearing in bovine feces were calcium oxalate, a few were potassium oxalate, and some contained both compounds. From 20 to 33% of the Ca in alfalfa is in the form of oxalate, a form which is poorly available to ruminants according to Ward et al. (1979).

The ARC (1980) adopted a value of 16.0 mg/kg body weight

per day for the fecal endogenous loss of Ca in cattle, a value similar to 16.4 mg/kg body weight per day, observed by Yoon and Evans (1981).

Phosphorus

Sheep fed on roughage diets can control intestinal P absorption very effectively to the amount needed to replace that secreted in the saliva and, in so doing, they favor the gut rather than the kidney as a pathway for excretion of excess dietary P (Scott and McLean, 1981).

In a review article, Cohen (1980) stated that the P in saliva is mostly inorganic phosphate and its concentration exceeds that of serum by a factor of at least 5 for sheep and cattle. Some generalizations about the P secretion in saliva were also stated. The first was that P output in saliva was dependent on P intake. The second was that salivary P concentration was directly related to plasma P concentration and varied inversely with saliva flow rate, which tended to keep the daily output of salivary P constant. The third was that salivary P was the most important source of P in the rumen in rangeland ruminant nutrition. Scott and McLean (1981) mentioned that a 40-kg sheep may secrete in excess of 5-6 g of P per day in saliva.

Tomas (1974) observed that wether sheep fitted with permanent bilateral re-entrant parotid duct cannulas would show an increase in urinary P excretion, which was proportional to

the fractional amount of secreted P diverted from the rumen to the blood. Urinary P excretion in sheep with bilateral parotid duct ligation, fed a P sufficient diet, increased 20- to 50-fold following ligation (Tomas and Somers, 1974). Phosphorus balance remained unaltered by either diverting the saliva from the rumen to the blood or ligating both parotid ducts (Tomas, 1974; Tomas and Somers, 1974). Thus, the salivary secretion of P to the gut seems to contribute towards maintenance of the P homeostasis in sheep (Tomas and Somers, 1974).

Poppi and Ternouth (1979) noted a large net secretion of P into the rumen and the first quarter of the small intestine and net absorption from the remainder of the small intestine in sheep. Stevenson and Unsworth (1978) observed a marked increase in the amount of P reaching the proximal duodenum relative to that ingested, and most of the element secreted into the gastrointestinal tract of sheep was reabsorbed. Cohen (1980) agreed that the main site of P absorption is the small intestine. He also stated that the absorption from the rumen and omasum is negligible and that there is no clear evidence to support absorption from the abomasum.

Cohen (1980) stated that the absorption of P consists of both active transport and passive diffusion. Towns et al. (1980) agreed with Cohen (1980) and showed some evidence for three sites for P absorption. When sheep were injected with ^{32}P, via the abomasum, the plasma clearance curves showed three

peaks of absorption. The first peak, probably the absorption from the duodenum or proximal small intestines was greatly reduced by high P intakes. However, Fox et al. (1978), using a Thiry-Vella loop of jejunum of pigs, demonstrated that the net absorption of phosphate increased with increasing concentrations of phosphate in the perfusion solution and that net absorption of phosphate was not saturated even at 50 mM P concentration.

The rates of apparent absorption and retention of P were increased by 1-OH-D_3 treatment (Braithwaite, 1978b) and by infusion of Ca directly into the bloodstream of sheep (Braithwaite, 1978a). Phosphorus retention was increased by Ca infusion, possibly as a result of the increased Ca retention.

Absorption of P was similar from an organic (phytic acid) and an inorganic (sodium phosphate) form of dietary phosphorus (Dutton and Fontenot, 1967) and from sodium orthophosphate, meat and bone meal and wheat bran as sources of P for sheep (Poppi and Ternouth, 1979). However, retention was higher from the inorganic form of dietary P in the former work.

Magnesium

A net secretion of Mg occurred in the upper small intestine of cattle (Perry et al., 1967; Greene et al., 1981). The absorption of this element was greatest in passage of digesta through the small intestine, with few changes occurring during the passage through the cecum and large intestine of calves

(Perry et al., 1967); however, Tomas and Potter (1976) stated that the absorption of Mg post-ruminally was insufficient to maintain normal Mg status in the adult ruminant animals. Little net change in Mg relative to an unabsorbed marker was found between the duodenum and ileum even for a diet containing a Mg concentration of 8 g/kg DM (Horn and Smith, 1978).

Stevenson and Unsworth (1978) observed a net absorption of Mg (31 to 41%) occurring before the proximal duodenum of sheep. Giduck et al. (1981) and Greene et al. (1981) found that the primary site of Mg absorption was preintestinally. Tomas and Potter (1976) were more specific than the previous authors saying that no significant absorption of Mg occurs from either the omasum or abomasum in sheep and that the reticulo-rumen is the main site of Mg absorption before the pylorus. In fact, Rayssiguier and Remesy (1977) provided some evidence that the absorption of Mg is related to volatile fatty-acid production.

Martens et al. (1978) and Brown et al. (1978), using pouches of dorsal rumen sac and isolated rumen epithelium of sheep, respectively, showed that Mg was transported against an electro-chemical gradient. They also showed that the absorptive mechanism was saturable with concentrations of Mg between 4 and 5 mM. Martens et al. (1978) also reported that the Mg transport was markedly reduced by lowering the temperature of the bathing solution.

Field et al. (1981) observed no effect of either changing the frequency of feeding or Mg supplementation on the absorption of Mg in sheep. However, Dutton and Fontenot (1967) noted higher absorption and retention of Mg in the group of sheep receiving .26% Mg compared to .13% Mg fed-group.

Giduck et al. (1981) reported that the level of soluble carbohydrate (3 vs 23%) in the diet did not affect Mg absorption, even though Fenner (1979) stated that a low pH would favor the Mg absorption.

Another factor that affects the absorption of Mg is the level of K in the diet. Both Greene et al. (1981) and Giduck et al. (1981) observed a decreased Mg absorption with high levels of K in the diet of steers and sheep, respectively.

Powley et al. (1977) reported that daily endogenous fecal Mg excretion was approximately 13% of the total fecal Mg output and was not affected by altering the herbage levels of Na and K. Chicco et al. (1972) estimated the endogenous fecal Mg excretion of sheep to be 2.33 mg/kg body weight daily. ARC (1980) has adopted a value of 3.0 mg/kg body weight for the endogenous fecal loss of Mg.

Potassium

Ward (1966) reviewed the K metabolism and concluded that ruminants and other herbivores normally consume amounts of K greatly in excess of their dietary requirements, which is probably no more than about .5% of the total daily ration.

Perry et al. (1967) reported a net secretion of K in the upper small intestines of calves and absorption of this element was greatest in passage of digesta through the small intestine. Both Greene et al. (1981) and Giduck et al. (1981) agreed that the main site of K absorption was from the intestine in sheep and cattle when low (.6% of DM) levels of this mineral element was fed. However, when higher levels (4.0 to 4.8% of DM) of K was fed, the pre-intestinal region was also an important site of absorption. Potassium absorption increased with increasing levels of K in the diet of sheep (Giduck et al., 1981) and steers (Greene et al., 1981).

Zinc

Absorption and retention of ^{65}Zn from an organic form, i.e., grown into young corn and rye plants (Neathery et al., 1975) and into spinach plants (Welch et al., 1977) were higher than from an inorganic form of this element. However, the relatively high absorption and retention from both forms of Zn in rats indicated that the incorporation of Zn into young forage protein is not of major practical importance. Evans and Johnson (1977) observed no difference between the absorption of intrinsic and extrinsic labelled corn and liver in rats.

Hardie-Muncy and Rasmunssen (1979) reported that the absorption of Zn from Zn salts was depressed by soybean-isolate when compared to casein as sources of protein for the rat.

Both Ca and phytate affect Zn absorption. According to Huber and Gershoff (1970), the effect of Ca on fecal excretion of Zn appeared to be entirely related to its effect on Zn absorption in the rat. However, Ca as ground limestone had no effect on absorption of Zn in the lactating cow (Kincaid, 1979). The data of Morris and Ellis (1980a) indicated that high dietary Ca per se reduced Zn bioavailability. High levels of phytate have been demonstrated to reduce Zn absorption (Lo et al., 1981a). Furthermore, an interaction between Ca and phytate was demonstrated by Morris and Ellis (1980a). On diets with 10-12 ppm Zn, the growth of rats was not affected by phytate: Zn molar ratios of 12 or less if the level of dietary Ca was .75% but was depressed at ratios greater than 6 if the level of Ca was 1.75%. Thus, Zn bioavailability to rats fed isolated soybean protein can be improved by fortifying it with Zn so that the phytate:Zn molar ratio is less than ten (Lo et al., 1981a). Low-phytate brans with phytate:Zn molar ratios of 8 or less were equivalent to zinc sulfate as dietary sources of Zn for growth of rats (Morris and Ellis, 1980b).

Evans and Johnson (1980) reported that either picolinic acid or its precursor, tryptophan, facilitates Zn absorption in rats; however, Flagstad (1981) reported no increase in absorption of Zn by picolinic acid supplementation to cattle. According to the latter author, picolinic acid is more easily decomposed by the ruminant stomach system than hydroxyquinolines. The latter compound when supplemented to cattle

suffering from Adema disease (Acrodermatitis enteropathica) caused an increase in intestinal ^{65}Zn absorption while picolinic acid had no such effect.

The quantities of Zn leaving the stomach were found to be significantly greater than those ingested in the feed by sheep (Grace, 1975; Stevenson and Unsworth, 1978). A significant net absorption of Zn from the small and large intestines was noted in sheep (Grace, 1975) and from the abomasum and lower small intestine in dairy cattle (Miller and Cragle, 1965). Smith and Cousins (1980) using the isolated vascularly perfused rat intestine technique provided evidence that the intestinal cell is a major site of regulation of Zn absorption. Using this same technique, Antonson et al. (1979) concluded that the distal ileum contained the greatest capacity for Zn absorption in the rat small intestine. The presence of the protein ligand of pancreatic origin did not increase Zn absorption in the duodenum when studied by this technique.

The retention of ^{65}Zn in rats, whether administered orally or intraperitoneally, was directly related to the levels of Zn fed; Zn loss was primarily in the feces (Huber and Gershoff, 1970). Indeed, Weigand and Kirchgessner (1980) showed a large difference between apparent and true absorption of Zn when increasing levels of this mineral element were fed to rats. This difference in apparent and true absorption rate was due to the endogenous Zn excretion which increased with increasing

levels of Zn in the diet. Thus, Weigand and Kirchgessner (1980) concluded that not only the absorptive but also the metabolic efficiency greatly contributed to Zn homeostasis and true efficiency of Zn utilization.

Copper

Wiener et al. (1978) studying the breed differences of sheep in Cu metabolism concluded that the large discrepancies between groups in response to oral Cu repletion were largely attributable to differences in the efficiency of Cu absorption.

Grace (1975) observed no net secretion of Cu into the forestomach and small intestine and observed a net absorption from the large intestines of sheep.

Water Soluble Mineral Element and Nitrogen

About 25% of the total DM in grasses and clovers was found to be water-soluble (Todd, 1961). This water-soluble fraction included more than 50% of the total Mg when the forage contained large amounts of Mg (.2% or more) in the DM. However, when smaller amounts of Mg were present in the herbage (less than .2% of the DM), the water-soluble fraction contained less Mg than the residue from the water-extraction procedure.

Of the total P present in hayed-off phalaris and clover plants, 60-83% was water-soluble and most of it was inorganic (Bromfield and Jones, 1972).

Glenn et al. (1980) measured the disappearance of N and S by the nylon bag technique. They reported that the soluble disappearance of the total N in tall fescue and orchardgrass showed an initial water extraction which was 46.4% and 31.7%, respectively. Water-extracted S determined by this same technique varied with the fertilization regime. On the average, 59.2 and 51.3% of the total S in the tall fescue and orchardgrass, respectively, was water-soluble. When only N fertilizer was applied, the soluble S dropped to about half of either the unfertilized or only S fertilized treatments for both grasses.

From the data of Playne et al. (1978b), after 12 hours (h) in nylon bags in the rumen, the disappearance (water-extracted, % of total) of DM, N, Ca, P, S, Mg, and K from four tropical hays was approximately 22, 24.5, 29.1, 34.2, 59.1, 66.4, and 80.6%, respectively.

The disagreement between the P solubility in the work of Bromfield and Jones (1972) and Playne et al. (1978b) is probably related to differences among plant species; i.e., from the total amount of P initially present in Medicago sativa, Stylosanthes humilis, Chloris barbata, and Heteropogan contortus, approximately 62, 47, 22, and 10% were solubilized

after 12 h in nylon bags in the rumen (Playne et al., 1978b).

Protein Degradation in the Rumen

Basal rations and protein supplements

Dietary protein ingested by ruminant animals is extensively degraded by microorganisms inhabiting their forestomachs. According to Tamminga (1979), experimental results, both *in vivo* and *in vitro*, show a varying degradation between individual amino acids. Part of the variation, particularly *in vivo*, must be attributed to inadequate measuring techniques.

Apart from solubility, structural differences, to a certain extent caused by disulfide bridges crosslinking the protein, may be important determinants of degradability (Nugent and Mangan, 1978). Among other factors influencing protein degradation are solubility of dietary protein, rate of passage of digesta through the forestomachs and the level of feed intake (Tamminga, 1979). The solubility of the protein of tropical pasture species determined with the Burroughs' solution (Aii and Stobbs, 1980) and that of heat-treated cottonseed meal determined with the McDougall's and Burroughs' buffers and with .02 N sodium hydroxide (Craig and Broderick, 1981) have shown conflicting results with *in vivo* degradation rates. In the latter work, ruminal protein degradation was more highly correlated with N solubilization in .02 N sodium hydroxide than in McDougall's and Burroughs' buffers. Furthermore,

Nugent and Mangan (1978) reported that differences in the rate of rumen hydrolysis of three proteins (casein, leaf protein, and bovine serum albumin) were dependent upon the presence of proteolytic enzymes and on the structure of proteins rather than their degree of solubility.

The influence of the level of feed intake on protein degradation as stated by Tamminga (1979) was demonstrated by Zinn et al. (1981) with two rates of concentrate feeding to 180-kg calves. Rumen protein degradations were 103, 85, 76, and 54% for casein-, soybean meal-, cottonseed meal-, and corn gluten meal-supplemented diets, respectively, when these supplemented diets were fed at the rate of 3 kg/day. When fed at the rate of 4 kg/day, rumen protein degradation estimates were 82, 39, 56, 39, and 30% for soybean meal, cottonseed meal, linseed meal, corn gluten meal and meat and bone meal, respectively.

Determination of protein degradation

In vivo techniques This involves sampling the contents from the abomasum or duodenum of the ruminant animal. Such techniques rely on the estimation of undegraded dietary N by subtraction of the microbial contribution from the total N present in the sample, once a correction has been made for the endogenous N (Orskov et al., 1981). The microbial contribution to the total N presented to the duodenum for digestion and absorption is generally estimated using ^{35}S, ^{15}N, diamino-

pimelic acid (DAPA), and nucleic acids (El-Shazly and Abou Akkada, 1972).

In situ technique Feeds contained in porous synthetic fiber bags are suspended in the rumen of surgically modified ruminants. The attraction of this method lies in the ability of research workers to follow sequentially the rate of protein degradation in the rumen (Wilson and Strachan, 1981). Several ways of selecting the appropriate incubation time are possible. Mathers et al. (1977) found that an incubation of 4-6 h gave the best prediction of in vivo degradation for the feeds (concentrates) studied. Orskov and Mehrez (1977) suggested determining the extent of degradation of the protein of roughage feeds when 90% of the digestible DM had disappeared from the bag, based on the assumption that 90% of digestible cellulose and digestible starch is digested in the rumen (Armstrong and Beever, 1969). A third possible approach is to calculate a rate constant of disappearance from a logarithmic plot of the proportion of N remaining in the residue (Mohamed and Smith, 1977).

Wilson and Strachan (1981) derived the predictive equations $Y_1 = 2.22 - 0.28\ X_1$ and $Y_2 = 2.27 - 0.27\ X_2$ for silage and hay, respectively; where Y = log (100 - degradability, %) and X = square root of the % CP in DM. A different approach to the problem was taken by Orskov and McDonald (1979). They devised a mathematical combination of the nylon bag procedure

and rumen retention time measurements on the same protein supplement. The potential degradability, p, related to incubation time, t, is given by the equation $p = a + b\ (1 - e^{-ct})$. The effective N degradability, P, can be calculated from the relationship $P = a + \frac{bc}{c + k}$, after obtaining the fractional outflow rate per hour, k.

The validity of the *in situ* technique for measuring the degradation of protein in the rumen was questioned by Mathers and Aitchison (1981). In their experiment, the extent of microbial contamination of feed residues increased linearly with time in the rumen. After 48 h in the rumen, nearly one fifth of the residual N in lucerne samples was of microbial origin.

Laboratory procedures *In vitro* methods, such as the measurements of ammonia and amino acid release on incubation with rumen inoculum diluted with either the Burroughs' buffer or McDougall's buffer (Aii and Stobbs, 1980; Broderick, 1978; Craig and Broderick, 1981) appeared to be attractive on the basis of handling a large number of samples. However, the rumen fluid test underestimated the breakdown of feed proteins relative to casein because of fixation of ammonia due to fermentation of carbohydrates present in the feeds (Chamberlain and Thomas, 1979). Furthermore, the rates of degradation did not correlate with *in vivo* observations. Craig and Broderick (1981) found that ruminal protein degradation was more highly

correlated with N solubilities in .02 N sodium hydroxide than in McDougall's and Burroughs' buffers.

Another possibility would be the use of an artificial saliva containing a neutral protease enzyme (Chamberlain and Thomas, 1979). Except for casein, the solubility of the proteins were variable in these protease tests.

Biological Availability of Mineral Elements

Partridge (1980) stated that there are some difficulties associated with the concept of availability. Firstly, there is confusion in terminology. Terms such as availability (true and apparent), utilization, digestibility (true and apparent), absorption, and retention continue to be used interchangeably or given different definitions by different authors. Secondly, there are two overlapping but different concepts of availability. Some consider availability to be a characteristic of a particular source of an element. Others consider it to be a measure of the metabolic state of the animal. For example, when estimating the mineral requirements factorially, it must be remembered that, on the one hand, the proportion of an element that can be extracted by digestion and absorption differs from one source to another; on the other hand, the amount that is absorbed and utilized depends on the metabolic demands of the animal.

Peeler (1972) defined biological availability as being

a measure of the ability of the element form under consideration to support some physiological process in relative numerical terms in comparison to a reference standard.

Basal feeds and mineral supplements

Some level of the macro- and micro-mineral elements essential for animals occurs naturally in various forms in most feedstuffs. In addition, minerals are sometimes added as supplements to feeds to meet dietary requirements. The mineral supplements are chiefly chemical compounds, processed natural ores and by-products from industrial processing, varying greatly in chemical purity and biological availability (Ammerman and Miller, 1972; Peeler, 1972).

Calcium Hansard et al. (1957) employed radioisotope procedures for the measurement of endogenous Ca and subsequent calculation of its net absorption. Digestibility studies showed that the true absorption was greater in the young than in the mature steers and that the difference due to age was greater than that due to Ca source itself. In fact, Younoszai and Ghishan (1979) suggested a change in the mechanism(s) for intestinal transport of Ca during animal maturation. They noted a lower net absorption and lower estimated bidirectional fluxes of Ca (micromoles/h/g wt of intestine) for a 6-week-old rat than in younger animals.

The values of true digestibility and biological availability of Ca for 15 organic and inorganic sources reported

by Hansard et al. (1957) are shown in Table 3. Differences in Ca availability were not considered large. Nevertheless, the sources could be classified into 3 groups with bone meal, monocalcium and dicalcium phosphates having the highest availability for Ca and the hay sources, the lowest in availability. The availability of Ca in ground limestone, defluorinated phosphate and calcium carbonate was intermediate. According to Ward et al. (1979), 20 to 33% of the Ca in alfalfa is in the form of oxalate, a form which is poorly available to ruminants.

Phosphorus The availability of P in dicalcium phosphate, bone meal, and Curacao rock phosphate was found to be all equal for beef heifers when feed intake, weight gain and the level of P in plasma were used as criteria for evaluating availability (Long et al., 1957). Ammerman et al. (1957) also found no difference among five sources of P (dicalcium phosphate, bone meal, soft phosphate, Curacao rock phosphate and defluorinated rock phosphate) for beef steers when P retention and plasma P level were measured. However, in a study with lambs, they found that soft phosphate and defluorinated rock phosphate were poorly utilized. Wise et al. (1961) confirmed these previous lamb results working with dairy calves. Among the 4 phosphates tested, soft phosphate had the lowest availability as measured by its ability to maintain the level of P in plasma.

Table 3. Biological availability of Ca from various sources for young and mature steers[a]

Calcium source	True absorption		Biological availability	
	Mature	Young	Mature	Young
	----------		%	----------
Calcium carbonate, C.P., reference	40	51	100	100
Bone meal (imported)	55	68	138	133
Calcium chloride, C.P.	53	60	132	120
Dicalcium phosphate, C.P.	50	64	125	126
Monocalcium phosphate, C.P.	56	61	140	120
Dicalcium phosphate, A	49	58	122	114
Dicalcium phosphate, B	38	56	95	110
Dicalcium phosphate, C	56	60	140	120
Dicalcium phosphate, D	51	60	127	120
Dicalcium phosphate, E	55	58	138	114
Defluorinated phosphate	40	55	100	108
Ground limestone	37	45	93	88
Alfalfa hay	31	41	78	80
Lespedeza hay	36	50	90	98
Orchardgrass hay	39	51	98	100

[a]Hansard et al. (1957).

Lofgreen (1960) utilized a radioisotope dilution technique to determine the true absorption of P present in several inorganic phosphate supplements and calcium phytate. By assigning dicalcium phosphate the value of 100, the biological availabilities of bone meal, soft phosphate and calcium phytate were 92, 28, and 66%, respectively. The P in calcium phytate was significantly less available than in dicalcium phosphate and bone meal. However, in an *in vitro* work, Raun et al. (1956) found calcium phytate to be as available to rumen microorganisms as highly available inorganic phosphates. Using this same *in vitro* rumen technique, Anderson et al. (1956) gave approximate ratings of the availability of P in dicalcium phosphate, bone meal, Curacao rock phosphate and soft phosphate as being 93, 28, 12, and 2% of a standard sodium and potassium phosphate. The low availability values for some of these compounds may be more related to the relative insolubility of these compounds in the *in vitro* medium than to availability itself; thus, this information should be interpreted with caution when extrapolating artificial rumen results directly to the animal (Peeler, 1972).

Lofgreen and Kleiber (1954) reported a high biological availability of P (94%) in alfalfa hay for sheep. According to Peeler (1972), this value should have been expected because good quality alfalfa contains little, if any, phytate P. The content of phytate and inorganic P of a number of feed-

stuffs is presented in Table 4.

Apparent P absorption and retention by lambs from poultry litter was 60% of that of dicalcium phosphate. Results from plasma inorganic P levels also confirmed this result when using the slope ratio technique (Tagari et al., 1981).

The accepted general order of rank in availability of the supplements of P for livestock is as follows: soluble phosphates such as sodium phosphate and monocalcium phosphate are approximately equal and have the highest biological availability, followed closely by dicalcium phosphate. Next come defluorinated rock phosphate and bone meal, then low fluorine rock phosphate and, finally, soft phosphate.

The P requirement for milk production was estimated by NRC (1978) based on the P content of milk and 55% P availability to the animal, while the ARC (1980) adopted the value of 58% for the coefficient of absorption of dietary P.

Magnesium Although the degree of availability differed, Mg from all magnesium oxide forms examined was available for absorption (Jesse et al., 1981). The differences noted in Mg recoveries measured by the increase in urinary excretion of Mg above baseline for 2 days following an oral administration of the test substance, may result from the lower rate of solubilization in rumen fluid of the unground versus ground magnesium oxides. Chicco et al. (1972) observed a true absorption of Mg from magnesium oxide of 75%. The

Table 4. Total, phytate and inorganic P content of feedstuffs[a]

Feedstuff	Total P	Phytate P	Inorganic P
	----------	%	----------
Alfalfa meal, 17% CP	.28	.01	.27
Barley	.34	.19	.15
Corn	.26	.17	.09
Corn gluten meal, 41% CP	.58	.35	.23
Corn meal, degermed	.10	.07	.03
Cottonseed meal, 50% CP	1.29	.92	.37
Cottonseed meal, 41% CP	1.07	.75	.32
Distillers dried grains w/sol	.77	.33	.44
Distillers dried solubles	1.43	.10	1.33
Milo	.31	.21	.10
Oats	.34	.19	.15
Peanuts	.33	.23	.10
Rice bran	1.67	1.44	.23
Rice polishings	2.72	2.42	.30
Sesame meal	1.27	1.03	.24
Soybean meal, 50% CP	.61	.37	.24
Soybean meal, 44% CP	.66	.38	.28
Wheat	.30	.20	.10
Wheat bran	1.37	.96	.41
Wheat middlings	.47	.35	.12

[a]IMC (1973).

availability of Mg in dolomitic limestone was only 28% of that in magnesium oxide for Beef steers (Gerkin and Fontenot, 1967).

The availability of Mg reported for hays, forages and grains for ruminant animals is summarized in Table 5. The availability of Mg in forages is low and ranges from 10 to 25% and that in grains and concentrates ranges from 30 to 40%. Stillings et al. (1964) observed that low N hays had apparent availability of Mg somewhat higher than high N hays.

Potassium Large differences in availability of K probably should not be expected in view of the solubility and rapidity of absorption of the usual forms of this element in the animal's diet (Peeler, 1972).

Zinc A net absorption of ^{65}Zn administered daily averaged 12% in mature cows, 20% in calves from 5-12 months and 55% in week-old calves (Miller and Cragle, 1965). When the total collection of feces methodology was employed, the authors found that the true absorption of Zn was 14% in mature cows and 28% in calves.

Availabilities of Zn supplied as chloride, sulfate, oxide, and carbonate were comparable in both calves and rats (Kincaid, 1979). The availability of Zn in metallic Zn dust to growing pigs was estimated to be about 30% greater than that from zinc oxide powder (Miller et al., 1981). O'Dell et al. (1972) observed that the Zn in plant seeds was less available

Table 5. Availability of Mg in hays, forages and grain for ruminants[a]

Researcher	Species	Feedstuff	Availability (%)
Stillings et al., 1964	Sheep	Low N hay	18-24
		High N hay	11-16
Field, 1967	Sheep	Mixed pasture	16-26
Lomba et al., 1968	Dry cows	Mixed hays	23.1
	Lactating cows	Mixed hays	27.8
Rook et al., 1962	Cows	Hay	23-26
Rook et al., 1962	Cows	Grain	37.5

[a]Cited in Peeler (1972).

than that in animal products, to rats and chicks.

In estimating the gross requirements for Zn of growing and mature ruminants, the values of .30 and .20, respectively, for the coefficient of absorption of this element had been adopted by the ARC (1980).

Copper Several Cu-containing compounds have been tested as dietary supplements of this element for ruminants. Lassiter and Bell (1960) reported greater blood uptake of radioactive Cu in sheep when ^{64}Cu as cupric chloride was given orally than when either cupric sulfate or cupric nitrate were administered. Copper from oxide needles was less well-

absorbed than the other forms of this element tested. In another phase of the same study, cupric carbonate was better utilized than either cupric oxide or cuprous oxide.

Givens and Hopkins (1978) used the mathematical formula of Suttle and McLauchlan (1976) to determine the availability of Cu in herbage. When the animal requirements for available Cu were compared with the calculated concentrations of available Cu in the herbage, they provided a possible explanation for the widespread hypocupremia experienced in these areas. Availability of Cu was very low, only 3.4 and 3.3% in two of these areas, as determined by this mathematical formula.

The ARC (1980), in calculating the total requirements for Cu, used the value of .06 for the coefficient of absorption of Cu for adult sheep, .04 for all ruminating cattle and .70 for the preruminant calf.

Determination of availability of mineral elements in vivo

Nutritionists use various methods for measuring mineral utilization. Here are a few of them:

Growth, milk production and feed efficiency Growth, milk production and feed efficiency have been used as criteria for determining Ca and P availability (Kincaid et al., 1981); P availability (Heinemann et al., 1957; Long et al., 1957; Wise et al., 1961; Cohen, 1972; Fishwick et al., 1977; Bass et al., 1981); Mg availability (Mathison et al., 1981); and

Zn availability (Weigand and Kirchgessner, 1977; Miller et al., 1981). These criteria are not entirely satisfactory. The primary reason is related to the fact that there is a great variation in growth rates of animals which prohibits obtaining accurate values without using large number.

Conventional digestibility and balance trials Digestibility and mineral balances are probably the most widely used techniques to measure mineral utilization by livestock. For instance, they have been used as methods for measuring P and Mg utilization in sheep (Tagari et al., 1981; Ammerman et al., 1957), and in cattle (Cohen, 1972; Fishwick et al., 1977; Bass et al., 1981; Moore et al., 1971), and Ca availability in rats (Armstrong and Thomas, 1952). The fact that some minerals are excreted back into the gut after being absorbed limits its value.

Radioisotope mineral elements Radioisotope dilution technique has been used by Lofgreen and Kleiber (1954), Hansard et al. (1957), Lofgreen (1960) and Lassiter and Bell (1960) for measuring the endogenous excretion of some specific mineral element. Therefore, the availability of a specific mineral element (Ca, Cu, P) to cattle and sheep could be calculated.

Lo et al. (1981b) found that Zn absorption determined with the stable isotope (^{70}Zn) was highly correlated with that determined by the radioactive isotope (^{65}Zn). The question of

whether an extrinsically labelled mineral element could be used for measuring the availability of mineral elements in feeds was studied by Evans and Johnson (1977) and Welch et al. (1977). Since extrinsic ^{65}Zn enters a common pool with intrinsic Zn, whole-body absorption of extrinsic ^{65}Zn can be used to obtain an accurate estimate of the availability of Zn in food (Evans and Johnson, 1977).

The hazards of contamination with radioactive material have to be taken into consideration when choosing a technique.

Blood profile Phosphorus, Mg and Zn blood levels, and alkaline phosphatase activity have been used by several authors (Ammerman et al., 1957; Bass et al., 1981; Brum et al., 1980; Cohen, 1973a,b; Heinemann et al., 1957; Kincaid et al., 1981; Long et al., 1957; Tagari et al., 1981; Miller et al., 1981; Wise et al., 1961) in attempts to differentiate between dietary sources of mineral elements and relate them to biological availability.

Bone calcification Bone ash test or bone calcification technique has been used with chicks (Corley et al., 1980); with rabbits (Heinemann et al., 1957); and with cattle (Brum et al., 1980; Cohen, 1973a,b; Wise et al., 1961). Armstrong and Thomas (1952) used a slight modification of the above technique by measuring the total Ca retained in the carcass of rats (slaughter technique) to estimate the availability of Ca in forage legumes.

The bone ash test is the technique developed to the finest point with chicks and is the one which appears to give the most reliable results for Ca and P availability (IMC, 1973).

Others The availability of some mineral elements such as Mg, Zn and Fe in feedstuffs may be determined by a technique which might be specific for that element. For instance, Jesse et al. (1981) measured the availability of Mg from magnesium oxide by the increase in urinary excretion of Mg above a baseline for 2 days following an oral administration of 100 g of the magnesium oxide supplement to cattle. Kincaid (1979) evaluated Zn availability in calves and rats as being the percentage increase of Zn in plasma with dietary supplementation of this element. The availability of Fe to chicks (Soevik et al., 1979) and to rats (Hunter, 1981) was measured by hemoglobin concentration (g/100 ml) in the blood when these animals were fed increasing amounts of Fe in their diets.

Measuring Availability of Nutrients by Laboratory Techniques

Investigators have searched for several ways to solve this problem. Six groups of techniques developed for some specific purpose are described below.

Fermentation with rumen microorganisms in vitro

The best known laboratory technique for the determination of digestibility of DM of forages is the one developed by Tilley and Terry (1963). It is sometimes used as a standard to which other laboratory techniques are compared. It is described as being a simple technique for the determination in vitro of the DM and/or OM digestibility of small (.5 g) samples of dried forages, involving two stages of incubation. In the first, a sample of dried forage is fermented anaerobically at 38 C in the dark with a mixture of 20% strained rumen liquor and 80% McDougall's buffer solution during 48 h. In the second stage, the residual DM from the first stage is incubated with .2% pepsin dissolved in .1 N HCl at 38 C for a further 48 h.

The variability due to the strained rumen liquor in the two-stage in vitro technique of Tilley and Terry (1963) was studied by Clark (1975) with a herbage and a mixture of grain and hay samples. The correlation coefficients between in vivo and in vitro digestibilities were higher for either the herbages or mixtures of grain and hay when the donor of the rumen fluid was fed hay than when fed a mixture of barley and hay.

Troelsen (1970) attempted to determine the energy value of the OM digested in vitro, by the difference between the energy in the substrate and that in the residue from a two-stage in vitro technique. The in vitro assay did not directly

reproduce the *in vivo* digestible energy levels over the entire quality range of the 102 hays studied. Exclusion from the regression of the hays containing less than 2 and more than 3 digestible kcal/g revealed that the *in vitro* assay could reproduce the in vivo digestible energy value. The chemical composition of the residue from the *in vitro* digestion (Tilley and Terry, 1963; Troelsen, 1970) and the animal feces were markedly different, especially with respect to their ash content. In fact, the digestibility of the ash *in vivo* was was 47% on the average for six tropical grasses compared with 71% *in vitro* (McLeod and Minson, 1974).

According to Osbourn and Terry (1977), the variation in the proportion of the apparently digested OM arising from cell walls severely limits the usefulness of chemical determinations and the single stage *in vitro* procedures as methods for predicting digestibility. A second stage using a solvent or enzyme to solubilize protein is invariably required in addition to an estimate of cell wall degradation.

Another laboratory technique which was developed by Burroughs et al. (1950) for studying cellulose digestion by rumen microorganisms was utilized by Anderson et al. (1956) for measuring the availability of P in feed supplements fed to ruminants and by Raun et al. (1956) for determining the availability of phytate P to rumen microorganisms. This artificial rumen technique is based on the concept that phos-

phate depleted rumen bacteria will rapidly digest cellulose only when supplied with adequate amounts of available P. However, caution should be used when ranking feed supplements which include relatively insoluble compounds. This technique seems to be very sensitive to the degree of solubility of the phosphates in the rumen liquor mixed with the artificial saliva (Anderson et al., 1956).

In vitro methods occupy an uneasy position between the generally fallible chemical methods and the direct animal estimates of the nutritive value of feeds for ruminants (Osbourn and Terry, 1977).

Solubilization with enzymes

Donefer et al. (1966) reported that % DM disappearance by a solution of .2% pepsin dissolved in .075 N HCl was found to be highly correlated with forage digestible energy intake potential as expressed by their nutritive value index (NVI) measured in vivo.

Results from in vitro experiments using cellulase enzymes have been reported for solubilizing DM of temperate grass species (Jones and Hayward, 1973; Jones and Hayward, 1975), of silages (Dowman and Collins, 1977), of grasses and brassicas (Allison and Borzucki, 1978), and of tropical forage samples (Adegbola and Paladines, 1977).

Jones and Hayward (1973) reported that the crude cellulase preparations from Trichoderma viride commercially avail-

able showed cellulase, hemicellulase, and proteolytic activity when tested on herbage and herbage polysaccharides. Jarrige et al. (1970) also reported that the cellulase preparations extracted not only cellulose, but also 14 to 20% of hemicellulose, 20 to 23% of the nitrogenous constituents and reduced the % of crude lignin from 8 to 12 units.

The original Jones and Hayward (1973) procedure used 200-mg dry sample incubated for 48 h at 40 C with 20 ml of a citric-phosphate buffer solution (pH 4.6) containing 6.25 g of Trichoderma viride cellulase enzyme per liter (1). This simple one-stage enzyme digestion technique was reported to give high correlations with in vivo DM digestibilities for a range of grass species. However, it digested much less DM than the in vitro technique described by Tilley and Terry (1963), and the DM digested in vivo. McQueen and Van Soest (1975) also confirmed these results.

Modifications of the Jones and Hayward (1973) technique were proposed by several authors (Jones and Hayward, 1975; Dowman and Collins, 1977; Allison and Borzucki, 1978; Roughan and Holland, 1977; Kellner and Kirchgessner, 1977; Clark and Beard, 1977).

Jones and Hayward (1975) reported that the correlation coefficients of the cellulase solubilization of the DM with in vivo and in vitro digestibilities of grasses was markedly improved by the pretreatment of the herbage sample with .2%

pepsin enzyme dissolved in .1 N HCl for 24 h at 40 C. This modified two-stage procedure of Jones and Hayward (1975) was reported by Terry et al. (1978) and Adegbola and Paladines (1977) not to be sufficiently accurate to predict the digestibility of the DM of legumes and tropical forages, respectively.

Dowman and Collins (1977) proposed grinding the samples through a .75 mm screen and increasing the concentration of cellulase in order to reduce the digestion time of the procedure of Jones and Hayward (1975) from 48 h to 24 h, while maintaining a high degree of correlation with *in vivo* DM digestibility. Allison and Borzucki (1978) proposed a modification in the pepsin pretreatment conditions (raising the temperature to 50 C and the acid concentration to .125 N) so that the cellulase values (disappearance of DM) were as high as those obtained using rumen liquor.

Roughan and Holland (1977) used a pretreatment stage with boiling neutral detergent acid (NDF) solution followed by washing with water and an exhaustive hydrolysis with cellulase enzyme. This exhaustive hydrolysis consisted of resuspending the residue in cellulase solution for an initial period of 5 h at 50 C, and then again with fresh cellulase solution for a further 18 h period at 50 C. Kellner and Kirchgessner (1977) also proposed a three-stage procedure with a pretreatment of 2 N HCl at 100 C for 30 min followed by incubations with cellulase for 24 h at 39 C and pepsin-HCl for another 24 h period.

Clark and Beard (1977) reported that the use of cellulase-amylase two-stage technique gave lower correlation coefficients with the two-stage rumen fluid *in vitro* digestion (IVDMD) than did the pepsin-cellulase technique.

Cellulase preparations in their current forms digest much less of the cell walls of grasses than does the mixed microbial inoculum of rumen liquor *in vitro*. Their specificity seems to make them less effective in the solubilization of the cell walls of legumes (Osbourn and Terry, 1977).

Nylon bag technique (*in situ* technique)

The principle of the *in situ* technique was first used to assess digestibility of leaves placed in a perforated brass capsule and fed to sheep, and later recovered either from the feces or after killing the animal (Reaumur, 1752, as cited by Hungate, 1966). Later, the *in situ* technique, as it is used today, was developed as a means of assessing the digestion of feeds (roughages and concentrates) within the rumen of cattle and sheep (Quin et al., 1938). At that time, the technique was called the silk bag technique. Quin et al. (1938) used silk threads to suspend the bags in the rumen while Balch and Johnson (1950) used nylon strings. Nowadays, the technique consists of suspending several bags, made of a porous synthetic fiber (polyester or nylon cloths, polypropylene filter fabrics), containing the feed samples, in the rumen of fistulated animals.

The technique is attractive since the rate of digestion (disappearance from the bag) can be sequentially correlated with the in vivo digestibility of similar material outside the bag.

Several authors have used this one-stage technique (Lindberg, 1981; Monson et al., 1969; Neathery, 1969; Van Keuren and Heinemann, 1962; Nocek et al., 1979; Van Hellen and Ellis, 1977; Crawford et al., 1978) while others used a second stage consisting of an incubation in .2% pepsin dissolved in .1 N HCl for 48 h at 39 C in the laboratory (Aerts et al., 1977; Playne et al., 1978a). This pepsin-HCl treatment permits a more effective washing and leads to a better repeatability (Playne et al., 1978a). Moreover, a second stage using a solvent or enzyme to solubilize protein is invariably required in addition to an estimate of cell wall degradation in order to predict the utilization of feedstuffs by a ruminant animal (Osbourn and Terry, 1977).

This technique has been used for studying the digestibility of DM of forages (Monson et al., 1969; Van Keuren and Heinemann, 1962), of DM of forages and concentrates (Lindberg, 1981), of DM of crop residues (Neathery, 1969), of forage cell walls (Lindberg, 1981; Playne et al., 1972, 1978a), of protein in forages and concentrates (Mehrez and Orskov, 1977; Mathers et al., 1977; Orskov and Mehrez, 1977; Mohamed and Smith, 1977; Orskov and McDonald, 1979; Wilson and Strachan,

1981), and of mineral element in forages (Glenn et al., 1980; Playne et al., 1972, 1978b; Randy et al., 1981).

The release of Ca, N, S, and P during the digestion of the seeds and pods of Stylosanthes humilis in the rumen up to 72 h was measured by Playne et al. (1972). Crude protein and P appeared to be selectively solubilized, but Ca was only slowly digested in both seed and pod. The release of Ca, K, Mg, N, Na, P, and S from 4 tropical hays during their digestion in the rumen up to 168 h was studied by Playne et al. (1978b). The proportions of elements removed during digestion were positively related to the initial concentration of the elements in the hays. High proportions of Mg and K were released within 24 h in all hays. Only about 60% of N, P, and Ca initially present was removed even after 168 h in the rumen. Amounts of elements remaining became constant for each element after 48 h and were for N, 6.0; Ca, 3.0; K, 1.0; P, .7; Na, .7; S, .5; and Mg, .3 g element Kg^{-1} DM initially present, even though cell wall digestion continued.

Glenn et al. (1980) studied the disappearance of N and S from orchardgrass and tall fescue while Randy et al. (1981) studied the disappearance of Ca from several forages. Both groups of workers studied the solubilization of mineral element during a short period (24 h) of incubation in the rumen. Glenn et al. (1980) found that the disappearance of S was related to changes in forage N and S components caused by changes

in the fertilization regime. Randy et al. (1981) reported that the rate of disappearance of Ca in the legume hay was 2.3 times as fast as that in grass hay, while corn silage Ca was least available to rumen microorganisms.

Several factors affect the digestibility of the samples in nylon bags in the rumen. The most important factors are: the diet of the rumen fistulated animal, the relation of sample size to bag size, digestion time, pore size of the synthetic fiber cloth.

The effect of the diet of the rumen fistulated animal on the digestibility of DM was demonstrated by Neathery (1969). He showed that the disappearance of DM values were higher when the fistulated steer was fed coastal bermudagrass diet than when it was fed alfalfa-orchardgrass diet. Van Keuren and Heinemann (1962) showed that the digestibility of samples was increased when the fistulated animal was fed alfalfa hay in dry-lot instead of being on alfalfa-orchardgrass pasture. Playne et al. (1978a) mentioned that their preliminary experiments indicated that it was necessary to include some grass hay in the diets in order to achieve optimal rates of digestion of the samples in nylon bags in the rumen.

The effect of sample size in relation to bag size was studied by Uden et al. (1974), who reported that increasing the sample size from 6.5 to 50 mg/cm^2 of bag surface area decreased guineagrass cell wall digestibility from 54 to 38%.

Lindberg (1981) reported that only small differences in DM disappearance from nylon bags were observed when the sample size was increased from 5 to 10 mg sample/cm^2 of bag surface area. Increasing the sample size to 15 mg/cm^2 significantly decreased the DM disappearance from 10 micron-pore size bags. For hay, the N disappearance was not affected by sample size; however, increasing the sample size from 10 to 15 mg/cm^2 decreased the N disappearance from barley and concentrates.

The length of time of digestion in the rumen is known to be a factor influencing the disappearance of DM from nylon bags. Neathery (1969) indicated that there was no increase in DM disappearance past 72 h or digestion in the rumen. A significant roughage x time interaction indicated that some roughages required a shorter time in the rumen than others to reach a maximum disappearance. Playne et al. (1978a) found that after 72 h digestion, amounts of cell walls of all species remaining in the bag were about 33.5 g/100 g original DM. A further period of digestion up to 168 h did not greatly increase fiber digestion. The cell contents solubilized were removed within the first 12 h or digestion. Little further removal of them took place after that time. Mehrez and Orskov (1977) studying the disappearance of barley in dacron bags reported that there were increases in DM and N disappearances associated with increasing the incubation time up to 15 h, after which there were small increases when the incubation time

was increased to 18 or 24 h.

The effect of the pore size of the cloth on the digestibility in nylon bags was studied by Uden et al. (1974) who found that using 53 micron-pore size bags gave higher digestibility of guineagrass than either 35 or 20 micron-pore size bags. The authors mentioned that lack of gas release from 20 and 35 micron bags limited digestibility.

Orskov et al. (1981) recommended paying attention to the following points in order to obtain consistent results when working with the nylon bag technique:

(1) More than 50 cm^2 of bag area/g DM of sample

(2) Long string attaching bag to the cannula cap (25 cm in sheep and 50 cm in cattle)

(3) Pore size of the cloth within the range 30-100 micrometers which is large enough to prevent accumulation of gas in the bags, but small enough to keep particulate losses to a minimum.

(4) Rumen conditions should be such that substrates are available for maximal microbial activity; therefore, it would not limit the digestibility of the sample inside the bag.

Chemical analysis and prediction equations

Fonnesbeck (1976) proposed a system for chemically separating the nutritive, partially nutritive and nonnutritive matter of feeds and foods. By this system, the nutritive matter was composed of soluble carbohydrates (sol. CHO), protein, fats and fatty acids, and soluble ash; the partially nutritive matter was made of the cell wall carbohydrates cellulose and hemicellulose; and the nonnutritive matter was composed of lignin, nonnutritive solvent extract and acid insoluble ash. Also, since feeds contain a wide range in concentration of the various chemical components, a model which involves the major digestible energy supplying components (sol. CHO, proteins, fats) and the diluting components (cellulose, hemicellulose, lignin) in their purest form can more accurately predict the digestible energy of feeds in general. However, Aerts et al. (1977), in a comparison of seven laboratory methods for predicting OM digestibility, concluded that neither the purely chemical procedures nor the tabular digestibility coefficients were sufficiently accurate for this purpose. Considerably better results were obtained with methods using living rumen microorganisms (two-stage in vitro technique and nylon bag technique).

Silica, in addition to being a nonnutritive matter, has a depressing effect on in vitro DM digestibility of legume and nonlegume forages as reported by Gupta and Pradhan (1975). From the regression equations developed by the authors, it

could be seen that one unit silica depressed about 1.40 units in digestibility in nonlegumes and about .6 units in legumes.

Berger et al. (1979) reported on a regression equation for predicting the effect of harvest date on the feeding value of corn stalklage. Digestibility of DM of the corn stalk decreased linearly with time. The regression equation to predict DM digestibility (Y) was Y = 63.22 - 1.93 X, where X is the week after corn silage harvest.

A regression equation to predict the availability of Cu in herbage to sheep was developed by Suttle (1974) and Suttle and McLauchlan (1976). Suttle (1974) described that a relationship between plasma Cu response of hypocupremic ewes (Y, mg/l) and cu intake (X, mg/d) after 21 days was Y = 0.087 X - 0.250. Then, Suttle and McLauchlan (1976) using Suttle's repletion technique demonstrated that the true availability of Cu (TA of Cu) predicted from responses in plasma Cu was related to the concentration of S in the herbage and to a S x Mo interaction by the following equation: log TA Cu = -1.153 - 0.076 S - 0.013 (Mo x S), where TA Cu is the true availability of Cu, and Mo and S are dietary concentrations as mg/kg DM and g/kg DM, respectively. This equation implies that S exerts a predominant and independent effect on Cu availability whereas Mo has a lesser and S-dependent effect. Responses to dietary S and S x Mo were exponential rather than linear, indicating that increments at the lower end of the normal ranges of concentra-

tions have relatively large depressing effects on Cu availability.

Fecal components as related to digestibility and consumption

Holloway et al. (1981) studied the fecal components related to DM intake and to DM digestibility. Those related to DM intake were: DM, N, CF, EE, NFE, ASA (acid soluble ash), IVDMD and Zn. Those related to DMD (dry matter digestibility) were: DM, N, CF, ash, NFE, ASA, IVDMD, CWC and Zn. The authors also concluded that fecal N indices do not have broad application, because the relationship between fecal N and forage intake or digestibility changes as season of year, N in forage and species consumed change. However, the addition of EE, CWC and DM to models containing N largely overcomes these problems.

Cohen (1974), studying the P nutrition of beef cattle, reported on the use of fecal and blood P for the estimation of P intake. Phosphorus intake (X_c) was related to total daily fecal P excretion (Y_c) by the equation: $Y_c = 2.442 + 0.289\ X_c$. In sheep, the relationship was $Y_s = 0.564 + 0.586\ X_s$ (Bromfield and Jones, 1970). Because the constants 2.442 and .564 will vary with the size of cattle and sheep, respectively, both variables (Y and X) in the equations should be expressed in units of metabolic body weight, so that the prediction equations then are: $Y_c = 0.051 + 0.294\ X_c$ and $Y_s = 0.033 + 0.536\ X_s$, for cattle and sheep, respectively; where, Y_c and Y_s are

daily fecal P excretion ($g/W^{.75}$) and X_c and X_s are daily P intake ($g/W^{.75}$) for cattle and sheep, respectively.

It is therefore possible to estimate P intake (X_c) from the total daily fecal excretion from the equation of Cohen (1974): $X_c = 3.401\ Y_c - 0.173$.

Plasma inorganic P concentration was related to P intake, but the relationship varied depending on the time of day at which samples were collected (Cohen, 1974).

Microscopic evaluation of forage digestion

Akin (1979) reviewed the microscopic evaluation of forage digestion by microorganisms. Using microscopy techniques, differences were found in plant anatomy and sites of lignification that affected the digestibility among species and plant parts. In addition to providing additional information on factors affecting forage digestion, microscopic techniques should be used for rapid comparisons of the rate and extent of fiber digestion.

Ward et al. (1979), using scanning electron microscopy and energy-dispersive X-ray techniques, described Ca-containing crystals in alfalfa and in feces of bovines fed this forage. Most intact crystals appearing in feces were calcium oxalate, a few were potassium oxalate, and some contained both compounds. The authors found that 20 to 33% of Ca in alfalfa is in the form of oxalate, a form which is poorly available to ruminants.

EXPERIMENTAL PROCEDURE

Nitrogen and Mineral Composition of Cattle Feeds and Crop Residues

Preliminary usage of existing procedures with ashing modifications

The N and mineral composition of 2 feeds, 2 crop residues, and 1 waste product were determined according to existing procedures. Some modifications were introduced in the standard ashing procedure. The common name of the feeds and crop residues along with the reference number (international feed number) and their descriptions are shown in Table 6.

Table 6. Name, reference number and description of samples used in the preliminary studies of N and mineral composition of feeds and crop residues

Common name of feed	Scientific name	Reference no. or int. feed no.	Description
Alfalfa hay	*Medicago sativa*	1-00-054	Alfalfa, hay, s-c, pre-bloom
Corn stover	*Zea mays*	1-02-776	Corn, aerial part wo ears, wo husks, s-c, mature
Dry poultry waste	-	5-05-587	Poultry, litter
Oat straw	*Avena sativa*	1-03-283	Oat, straw
Soybean meal	*Glycine max*	5-04-604	Soybean, seeds, solv-extd, grnd

Nitrogen and phosphorus The analyses of N and P were determined according to the procedure of Williams and Twine (1967). Nitrogen was also determined by the macro-Kjeldahl procedure described by the A.O.A.C. (1965). For the determination of N and P in the procedure of Williams and Twine (1967), 200 to 400 mg samples were weighed depending on the expected N and P content of the sample. Samples were digested in micro-Kjeldahl racks (LABCONCO) using 5 ml of a digestion mixture composed of concentrated sulfuric acid, potassium sulfate (10% w/v) and a small amount of selenium powder (.1% w/v). After the digestion and subsequent dilution to 100-ml with distilled water, N was determined colorimetrically by the reaction of ammonium with phenol and hypochlorite using a Technicon AutoAnalyzer (Technicon Corporation, Chauncey, NY). Phosphorus was determined colorimetrically by its reaction with ammonium molybdate and ascorbic acid reagents, making use of the same apparatus.

Ashing procedure The ashing temperatures used were: 625, 600, 550, and 500 C. The length of time that the samples remained in the muffle furnace at the above temperatures were: 4 h and 1.5 h (1 hour and 30 minutes). The only factorial combination of temperature and time not used was 600 C and 4 h. Minerals (Ca, Mg, K, Zn, Cu) were analyzed after the ash had been solubilized (boiled) in 3 N HCl on a hot plate for 5-10 min. One-gram-sample was employed and the solubilized ash

transferred and diluted to 100 ml in a volumetric flask with deionized water.

Calcium, magnesium The analyses of Ca and Mg were conducted using a Perkin-Elmer atomic absorption spectrophotometer, Model 460 (Perkin-Elmer, Norwalk, CT), equipped with hollow-cathode lamps specific for the elements being determined. The dry ash method of digestion of samples was employed, as described before. Calcium was analyzed with the addition of 1% lanthanum (La) to the final solution (Heckman, 1967, Perkin-Elmer analytical methods, 1973). Magnesium was analyzed without the addition of La (Heckman, 1967).

Potassium Potassium was analyzed in the same dilution as used for the Mg analysis, according to the procedure described in the Perkin-Elmer analytical methods (1973).

Zinc and copper Zinc and copper were determined according to the procedure described by Heckman (1968) and the Perkin-Elmer analytical methods (1973).

Final adopted procedures

Eight forage feeds and 11 crop residues were selected for N and mineral analyses and potential utilization within the digestive tract of ruminants. The name of the feeds and crop residues along with the reference number (Int. Feed no.) and their descriptions are presented in Table 7. It includes 1 forage feed and 2 crop residues listed in Table 6. Later, another set of 2 forage feeds and 2 crop residues were selected.

Table 7. Name, reference number and description of samples used in determining the potential utilization of DM, N and minerals by the cellulase and nylon bag techniques

Common name of feed	Origin of sample	Scientific name
Soybean stover[a]	Iowa	Glycine max
Soybean stalks[b]	"	"
Soybean leaves[b]	"	"
Soybean pods[b]	"	"
Corn stover	"	Zea mays
Corn stover[a]	"	"
Corn stover silage[a]	"	"
Corn husks	"	"
Cornstalks	"	"
Corn leaves	"	"
Oat straw	"	Avena sativa
Alfalfa hay	"	Medicago sativa
Alfalfa hay	"	"
Alfalfa, 2nd cut	"	"
Reed canarygrass	"	Phalaris arundinacea
Smooth bromegrass	"	Bromus inermis
Tall fescue	"	Festuca arundinacea
Corn silage[a]	Brazil	Zea mays
Elephant grass silage[a]	"	Pennisetum purpureum

[a]Samples from digestibility studies with sheep.

[b]Composited samples of 5 varieties (Wayne, Amsoy, Corsoy, Woodward, Calland) and 2 years (1974 and 1975).

Reference no.	Description
1-04-567	Soybean, straw
1-04-565	Soybean, stems
1-04-564	Soybean, leaves
1-04-563	Soybean, pods
1-02-776	Corn, aerial part wo ears, wo husks
1-02-776	Corn, aerial part wo ears, wo husks
3-02-835	Corn, aerial part wo ears, wo husks, ensiled, mature
1-02-784	Corn, husks, s-c, mature
1-02-795	Corn, stems, s-c, mature
1-02-787	Corn, leaves, s-c, mature
1-03-283	Oat, straw
1-00-071	Alfalfa, hay, s-c, mature
1-00-054	Alfalfa, hay, s-c, pre-bloom
2-00-179	Alfalfa, aerial part, fresh, pre-bloom, cut 2
2-01-113	Canarygrass, reed, aerial part, fresh
2-00-963	Brome, smooth, aerial part, fresh
2-01-889	Fescue, alta, aerial part, fresh
3-08-154	Corn, aerial part, w ears w husks, ensiled, mature
3-03-170	Napiergrass, aerial part, ensiled

They are listed in Table 8.

The laboratory procedures used to determine the composition of these samples were as follows:

Nitrogen The N content of the samples described in Table 7 was determined by the micro-Kjeldahl procedure (A.O.A.C., 1965), using 200 mg samples digested in sulfuric acid, potassium sulfate, and copper sulfate. Following cooling and after the addition of sodium hydroxide, they were distilled in boric acid-containing flasks and then titrated with .1 N HCl. The samples described in Table 8 were digested as described above; however, the N concentration was measured colorimetrically by the reaction of ammonia and salicylate using a Technicon AutoAnalyzer (Hambleton, 1977).

Dry matter and ash Samples were weighed into tared porcelain crucibles for the DM determination (105 C, 12 h). The dry sample was then ashed at 550 C for 1.5 h. All mineral elements were analyzed from this ash.

Phosphorus The P content of the samples described in Tables 7 and 8 was determined colorimetrically utilizing its reaction with the molybdovanadate reagent according to the A.O.A.C. (1965) procedure, using a UV-Visible spectrophotometer, Model 634 (Varian Techtron, Palo Alto, California) and DB-G Grating spectrophotometer (Beckman, Palo Alto, CA), respectively. The dry ash method was used to prepare the samples. The ash from the 3 g sample was solubilized (boiled) with 5 ml of 3 N HCl solution and a few drops of nitric acid,

Table 8. Name, reference number and description of samples used in determining the potential utilization of DM, N, and minerals by the nylon bag technique

Common name of feed	Origin of sample	Scientific name	Reference no.	Description
Alfalfa hay	Iowa	Medicago sativa	1-00-059	Alfalfa, hay, s-c, early bloom
Corn stover silage[a]	Iowa	Zea mays	3-02-835	Corn, aerial part wo ears, wo husks, ensiled, mature
Corn cobs	Iowa	Zea mays	1-02-782	Corn, cobs, grnd
Whole plant corn silage[a]	Iowa	Zea mays	3-08-600	Corn, aerial part w ears, ensiled, not well-eared

[a]Silages fed to fistulated animals.

then transferred to 25-ml volumetric flasks. These solutions were diluted just before the analysis, when necessary.

Calcium, magnesium and potassium These mineral elements were determined in samples according to the procedures of Heckman (1967). The samples were prepared as in the P analysis previously described. Two atomic absorption spectrophotometers were used. A Varian Techtron, Model AA6 was used to measure these mineral elements in samples described in Table 7, while a Perkin-Elmer, Model 460 was used to measure these mineral elements in samples described in Table 8. Calcium was determined when the final dilution contained .1% La.

Zinc, manganese and copper The analyses of these micro-elements in the samples were done according to Heckman (1968). The samples were prepared by the dry ash method as in the P determination. All analyses were performed either using a Varian Techtron or a Perkin-Elmer atomic absorption spectrophotometer as described for the Ca analysis.

In all cases, the atomic absorption apparatus was equipped with an air-acetylene burner and hollow-cathode lamp appropriate for the mineral element being analyzed.

IVDMD All samples shown in Table 7 were analyzed for *in vitro* DM disappearance by a modified version of the Tilley and Terry (1963) two-stage technique. The rumen fluid was collected from 3 sheep fed on elephant grass silage and concentrate.

Modification of the IVDMD

Two trials were conducted using the *in vitro* technique reported by Tilley and Terry (1963). A modification of the washing of the residue was proposed. Instead of the standard wash with hot water, the residues were submitted to "exhaustive washing". This consisted of sedimenting the contents remaining in the digestion tubes by centrifuging, discarding the supernatant fluid, resuspending the solids in glass distilled water, shaking for 30 min, centrifuging and discarding the fluid again. These steps of the procedure were repeated twice. After the third time, the solids were resuspended in water, the liquid was filtered through ashless filter paper with addition of hot distilled water.

Samples described in Table 6 were randomized with 4 treatments and 3 replications in trial 1. The treatments were:

1. One-stage digestion (48 h) with standard washing of residue
2. One-stage digestion (48 h) with "exhaustive washing" of the residue
3. Two-stage digestion (48 + 24 h) with standard washing of residue
4. Two-stage digestion (48 + 24 h) with "exhaustive washing" of the residue.

In treatments 1 and 2, samples weighing approximately 350 mg DM were fermented with 35 ml of a mixture of rumen

fluid and artificial saliva (1:4) for 48 h at 39 C in the dark (one-stage digestion). However, in treatments 3 and 4, the residues from the fermentation period similar to that described for treatments 1 and 2, were incubated with .2% pepsin-HCl for 24 h at 39 C (two-stage digestion). At the end of either the fermentation period (treatment 1) or the incubation with pepsin (treatment 3), the supernatant fluid was filtered through ashless filter paper, and washed with hot water. In treatments 3 and 4, the residues from either the one-stage or the two-stage digestion procedure were submitted to an "exhaustive washing" as described previously.

The residue was then dried at 80 C to constant weight and ashed at 550 C for 1.5 h, in order to determine the undigested dry weight and the % of ash in the dry residue.

In trial 2, the same treatments were used, except that treatment 2 was omitted.

Solubilization with Enzymes

Measurement of activity of cellulase enzymes

Two trials were conducted to test the activity of fungal cellulase enzymes. In trial 1, cellulase from *Trichoderma viride*, purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN) was determined according to the procedure of Jones and Hayward (1975). The activity of the enzyme was

measured by the ability of the cellulase to solubilize the DM of the following substrates: corn stover, soybean meal and cellulose, by incubation of 300 mg of substrate with concentrations from 0 to 480 mg of enzyme dissolved in 30 ml of a pH 4.8 citrate phosphate buffer (50.3% .1 M citric acid and 49.7% .2 M Na_2HPO_4) at 40 C for 24 h. At the end of the incubation period, the supernatant fluid was filtered through filter paper and the residue dried at 80 C to constant weight.

In trial 2, conducted in Brazil, cellulase donated by Biobras (Montes Claros, MG, Brazil) was used to digest samples of smooth bromegrass described in Table 7. Zero, 3, 6, and 12 g cellulase per liter of citrate phosphate buffer (pH 4.8) were used to solubilize samples during 48 h at 40 C following a pretreatment with .2% pepsin dissolved in .1 N HCl for 24 h at 40 C. Three-gram samples and 300 ml of buffer and buffer + enzyme were incubated in 500-ml Erlenmeyer flasks using an incubator with the temperature controlled thermostatically. The residue was dried and analyzed for ash content.

Measurement of solubilization of feed samples with 4 different enzymes

Three hundred milligrams of 5 different samples listed in Table 6 were incubated either with buffer alone or buffer + 180 mg of enzyme at 40 C for 48 h. The procedure employed was similar to that described by Jones and Hayward (1973) for the solubilization of DM of herbages, except that more than one

type of enzyme was used and the sample size and buffer volume and enzyme weight were all increased, but the same proportion of buffer and enzyme to substrate recommended by the cited authors was maintained.

Cellulase enzyme from Trichoderma viride (Boehringer Mannheim Biochemicals, Indianapolis, IN), cellulase WT from Aspergillus niger, protease WT and amylase WT recommended for waste-water treatment by Gist-Brocades nv (Delft, Netherlands) and donated by G. B. Fermentation Industries Inc. (Des Plaines, IL) were used in this trial.

A factorial combination of treatments in a completely randomized design, composed of 6 chemical treatments (4 enzymes and 2 buffers) and 5 different samples was used in this trial. All treatment combinations (30) were run in duplicate.

Both cellulase enzymes were dissolved in citrate-phosphate buffer (pH 4.8) while protease WT and amylase WT enzymes were dissolved in a pH 7.0 phosphate buffer (50% .5 M KH_2PO_4 and 50% .5 M K_2HPO_4).

At the end of the incubation period, the supernatant fluid was filtered through ashless filter paper and the residue was dried to constant weight at 80 C. Then, the dried residues were weighed and ashed in porcelain crucibles at 500 C for 1.5 h.

Effect of pretreatment on the solubilization with cellulase enzymes

Two trials were conducted testing the effect of pretreatments on the solubilization of samples with cellulase enzymes.

Trial 1 In trial 1, both alfalfa hay and corn stover described in Table 6, received one of the following pretreatments: none, H_2O, citrate-phosphate buffer (pH 4.8), .001 N HCl, .01 N HCl, .1 N HCl, and 2 N HCl for 24 h at room temperature. Samples that received one of these pretreatments were incubated either with citrate-phosphate buffer or 6.25 g/l of cellulase WT dissolved in this buffer, for 24 h at 40 C. One-gram sample and 100 ml of buffer or cellulase-buffer solutions were digested in screw cap polyethylene bottles as incubation vessels.

A factorial combination of treatments in a complete randomized design was employed using 7 pretreatments, 2 treatments (buffer and buffer + cellulase enzyme), and 2 samples (alfalfa hay and corn stover). All 28 treatment combinations were run in duplicate.

At the end of the incubation period, the contents of the bottles were filtered through filter paper, dried and ashed as described previously.

Trial 2 In trial 2, both alfalfa hay and corn stover were pretreated either with .1 N HCl or .2% pepsin dissolved in .1 N HCl for 24 h at 40 C. The main treatment was increas-

ing levels of T. viride cellulase added to either screw cap plastic bottles or 50-ml test tubes which were used as incubation vessels. All samples were treated with either 0, 6.25, 12.5, or 25.0 g/l of the enzyme.

A factorial combination of treatments in a complete randomized design composed of two separate tests which were run at the same time. Fifty-ml test tubes each containing 400-mg substrate, 40 ml buffer and one of the levels of enzymes were used in test 1 while the polyethylene bottles containing 1000 mg sample and 100 ml buffer and all levels of enzymes were utilized in test 2. The residue of the digestion from test 1 was dried and analyzed for N and P after digestion with sulfuric acid in the micro-Kjeldahl apparatus as described in the previous section. The residue from test 2 was dried, ashed, and analyzed for Ca, Mg, K, Zn, Cu using a Perkin-Elmer atomic absorption spectrophotometer as described in the same section. All treatments were run in duplicate within each test. For the statistical analysis of the DM disappearance, both tests were considered as one trial in a randomized complete block design.

Measurement of solubilization with cellulase with pepsin pretreatment

Samples described in Table 7, except corn silage and elephant grass silage, were used in these studies (two trials). A two-stage technique for the digestion of samples with cellulase enzyme, similar to that described by Jones and Hayward

(1975), was used. A 24-h pretreatment period at 40 C, with .2% pepsin-HCl, was followed by a 48 h incubation period at 40 C, using about 3 g samples and 300 ml of citrate-phosphate buffer (pH 4.8) containing 6.25 g/l of cellulase (Biobras, Montes Claros, MG, Brazil). Samples were enclosed in nylon bags, made from nylon cloth used in the clothing industry, and incubated in 500-ml Erlenmeyer flasks. Nylon bags were sewed with nylon thread using a sewing machine. Samples were washed twice in deionized water after the pretreatment period and at least three times after the incubation with cellulase. The washing procedure was stopped when the water remained clear upon its addition and hand-shaking the flask.

Bags containing samples and residues were dried in a 65 C oven for 72 h before and after incubation, respectively. After 200 mg of the residue had been wieghed out for N determination, the DM and ash were determined by weighing the remainder of the residue into tared porcelain crucibles and drying at 105 C for 24 h and ashing the dried residue at 550 C for 1.5 h. Mineral elements and N were determined in the residue by the same procedures used to measure them in feeds as described in the nitrogen and mineral composition of cattle feeds and crop residues section.

Nylon Bag Technique

Preliminary test

Alfalfa hay and corn stover feed samples described in Table 6 were used to test the *in situ* technique using a 20-micron pore size nylon cloth to make bags (6 x 6 cm internal dimensions) which would hold 3 g of sample. Bags were sewn with nylon thread using a sewing machine. Sample weight per unit cloth area was 41.6 mg/cm^2.

Digestion in situ Four nylon bags with three grams of each feed sample were suspended in the rumen of a fistulated cow fed an alfalfa hay and corn grain diet. The fistulated animal had free access to water and a block of trace-mineralized salt. The description of the alfalfa hay (sample no. 12) fed to the fistulated cow is presented in Table 7.

The samples remained in the rumen for 48 h before they were washed in running water to remove adherent rumen feed particles. The bags containing the residue from the rumen digestion were then incubated with .2% pepsin dissolved in .1 N HCl for 24 h at 39 C. After the incubation, the residues were washed at least 3 times with glass distilled deionized water, dried in an oven at 80 C until a constant weight was reached. All bags containing the samples had been dried and weighed before they were suspended in the rumen with a nylon fishing line.

Digestion in situ time assay

Digestion of alfalfa hay and corn stover samples was measured after 12, 24, 48, and 72 h in the rumen followed by a 24-h incubation period in pepsin-HCl, according to the same procedure described above (Aerts et al., 1977; Playne et al., 1978a). In this trial, only 3 replications (bags) per treatment were used. All other details of the procedure were similar to that of the experiment described above. After 200 mg of the residue from each bag were weighed for N and P analysis, the remainder of the residue was weighed into porcelain crucibles for the determinations of ash, Ca, Mg, K, Zn, and Cu.

Three additional trials were conducted in Brazil with a new cloth (Nitex cloth) purchased from Tetkco Inc. (Elmsford, New York). This cloth had a pore size of the same diameter (20 micrometers) as the one used in the previous trials; however, it was lighter in weight than the cloth used previously. One disadvantage of this cloth in relation to the first one used was that it tears easily.

The bags, 6 x 6 cm internal dimension, were made and sealed with an electrical resistance heat sealer (Polistar, Sao Paulo, SP, Brazil), normally used for making plastic bags. The bags held 3 g air-dry samples, with a ratio of sample weight to unit cloth area of 41.6 mg/cm^2 (Playne et al., 1978a). Three, five, and four replications were used in trials 1, 2,

and 3, respectively. The bags containing the samples (alfalfa hay and corn stover) remained in the rumen of the fistulated steer for 24, 48, and 72 h. Following the removal of the bags, the residue was incubated in .2% pepsin-HCl for 24 h at 39 C.

The diet of the fistulated animal was 20 kg elephant grass silage (25% DM) and 2 kg concentrate in the first trial and 20 kg whole plant corn silage (30% DM) and 2 kg concentrate in trials 2 and 3. Silage was restricted to 10-15 kg per day during the experimental period, depending upon the rumen fill.

Bags containing samples and residues were dried for 72 h in a 65 C oven and weighed before and after the two-stage digestion, respectively. The residue was analyzed for ash, N, and minerals by the same procedures described for the analysis of feeds in the nitrogen and mineral composition of cattle feeds and crop residues section.

Measuring the digestion of samples in situ

A two-stage technique was used for measuring digestibility in situ similar to that described by Aerts et al. (1977) and Playne et al. (1978a). The first step of the procedure consisted of digesting samples (3 g) in nylon bags (6 x 6 cm, internal dimensions), suspended in the rumen of a Holstein-Zebu crossbred rumen fistulated steer during 48 h. The diet of the fistulated animal was 15 kg whole plant corn silage and 2 kg of concentrate. The second step of the procedure consisted of washing the bags containing the residue from the rumen di-

gestion and incubating them in .2% pepsin-HCl for 24 h. This technique was repeated in two consecutive periods with the same 19 samples shown in Table 7.

After the second stage of the digestion, the bags containing the residues were thoroughly washed in deionized water. Then they were dried in an oven at 65 C for 72 h and weighed. Bags containing small pieces of plastic (3 g) were used as blanks to measure the entry of rumen particles into the bags during digestion. The weight loss from bags was assumed to be either water-soluble or digested by rumen microorganisms and pepsin, and therefore, potentially available to the animal.

Dry matter, ash, nitrogen, and minerals were determined in the residue by the same procedures used to measure them in feeds as described in the nitrogen and mineral composition of cattle feeds and crop residues section.

Pepsin treatment

Alfalfa hay and corn stover samples were submitted to either the one-stage (Playne et al., 1978b) or the two-stage (Aerts et al., 1977) nylon bag technique. The basic difference between the two was the inclusion of an incubation with .2% pepsin-HCl as the second stage of digestion in the latter technique, which the former procedure did not include. The length of the digestion time in the rumen was fixed at 24, 48, and 72 h, with four replications in a complete randomized factorial design. A 500-Kg Holstein-Zebu crossbred rumen-

fistulated steer was fed 20 kg corn silage and 2 kg concentrate, except during the collection period (experimental period) when the corn silage feeding was reduced to 10-15 kg per day depending on the rumen fill.

Bags containing the samples, residues and small pieces of plastic (blanks) were dried in an oven at 65 C for 72 h, and weighed. The loss of DM, ash, Ca, P, Mg, K, Zn, Mn, Cu from the bags were assumed to be potentially available nutrients for the rumen microorganisms and the host animal.

Dry matter, ash, nitrogen and mineral analysis were performed on the residues by the same procedures described previously for the analysis of feeds.

Losses of particulate matter and water soluble nutrients from bags

Inherent losses of particulate matter through the bag cloth and losses of water-soluble DM, N, and minerals from samples contained in nylon bags were measured in six small experiments. Four of them were conducted at Iowa State while two of them were conducted in Brazil.

The samples and the nylon cloth used in the first four trials have been described previously. In trials 5 and 6, dacron polyester cloth, described by Weakley et al. (1983), having an average pore size of 52 micrometers was used.

In trials 1 and 2, the bags (6 x 6 cm internal dimensions), containing 3 g of either alfalfa hay or corn stover, were sewn

with nylon thread using a sewing machine. In trials 3 and 4, the same size of bags were made and sealed with an electrical heat sealer. Similar samples used in trials 1 and 2 were also used in trial 3. In trial 4, 15 of the 19 samples described in Table 7 were used. Alfalfa hay, corn stover silage and ground corn cobs described in Table 8 were used in trial 5 while only alfalfa hay presented in the latter table was used trial 6.

In trials 5 and 6, the bags (8 x 9 cm internal dimensions), containing 3 g of samples in trial 5 and either 3, 1.5, or .75 g of alfalfa hay in trial 6, were sewn with polyester thread using a sewing machine. Seams were glued with Instant Vinyl glue (Sioux City, IA) to prevent particulate loss through the needle holes.

Each bag was placed in a 250-ml Erlenmeyer flask containing 150 ml of deionized water, and shaken for 8 h in trials 1, 2, and 5. In trials 3 and 4, 500-ml flasks containing 200 ml of deionized water were used. In trial 6, samples were shaken for 12 h. The water was changed every two hours in the first 5 trials. In trial 6, water was changed after 1, 3, 6, and 12 h in the shaker. A thymol crystal was added to all flasks during extraction to inhibit fermentation. At the end of the 8-h extraction in the first 5 trials and 12-h extraction in trial 6, feed particles which passed into the water were removed by filtration through a tared Whatman no. 41

filter paper. In trials 3 and 4, another brand (Ederol) of ashless filter paper was used. In the first 4 trials, the feed particles from the total extraction period were filtered altogether through the same filter paper. In trials 5 and 6, the feed particles which passed out of the bags at each time that the water was changed was filtered individually through filter paper.

The residues from the 8-h extractions with water in the first 5 trials were analyzed for DM, N, ash, and minerals (Ca, P, Mg, K, Zn, Mn, and Cu). The difference between the total amount of these elements present initially and that found in the residue was assumed to be the amount that was water-soluble. Thus, they were called water-soluble DM, ash, N, and minerals.

The disappearance of DM during digestion in nylon bags in the rumen and the total DM loss during extraction with water were corrected for losses of particulate matter through the bag cloth according to the following equation adapted from Playne et al. (1978a):

$$\% \text{ disappearance of DM} = 100 - \frac{R - E}{S(1 - C)} \times 100 \text{ , where}$$

R = DM weight of undigested residue; E = addition of DM from rumen contents to a bag containing small pieces of hard plastic (blank); S = DM weight of sample added to bag; C = correction factor (particulate matter lost as % of original DM added to the bag/100).

The disappearance of any element (N, Ca, P, Mg, K, Zn,

Mn, Cu) from the bags in the rumen or extracted in water can be calculated from the following equation:

$$\%\ \text{disappearance of element} = 100 - \frac{(R - E)B}{(S(1 - C))A} \times 100 \quad ,$$

where B = % element in the undigested residue; A = % element in the original sample, and R, E, S, and C as defined above.

The basic assumptions for the correction of the original weight put in the bags were that the particulate matter which passed through the cloth and the original sample have a similar chemical composition and that the fraction of the initial DM weight lost does not contribute to the undigested residue weight.

Size of the bag

The effects of varying the sample dry weight:bag surface area ratio on the disappearance of nutrients from bags incubated in the rumen of cattle were studied in two trials.

Trial 1 Samples (soybean pods and 2nd cut of alfalfa) weighing approximately 3 g were placed in either 6 x 6, 6 x 9, or 6 x 12 cm (internal dimensions) bags. Bags were made of Saatifil nylon (Tenyl, Sao Paulo, SP, Brazil), having a pore size of 30 micrometers. Then they were closed by a heat sealer after the insertion of the sample. Approximately 72, 108, or 144 cm^2 of bag surface area was exposed in the rumen, yielding a sample dry weight:bag surface area ratio of about 40, 30, or 20 mg sample/cm^2.

Two bags of each size of each sample were incubated for 48 h in the ventral sac of the rumen of two rumen fistulated steers. On removal, the bags were washed and the residues analyzed for DM, N, ash, and minerals according to the procedures described previously.

Trial 2 Samples (alfalfa hay, and corn stover silage) described in Table 8, weighing approximately 3 g were placed in either 6 x 6, 8 x 9, or 9 x 16 cm (internal dimensions) bags. Bags were made of dacron polyester (N. Erlanger, Blumgardt and Co., Inc., Broadway, NY) having an average pore size of 52 micrometers. Approximately 72, 144, or 288 cm^2 of bag surface area were exposed in the rumen, yielding a sample dry weight: bag surface area ratio of about 40, 20, or 10 mg sample/cm^2.

One size bag of each sample was placed in the ventral sac of the rumen of two rumen fistulated steers. The samples were incubated for either 12, 24, or 48 h in 3 consecutive 2-day periods. Samples were introduced into the rumen at different times, so that all could be taken out at the same time, 48 h after the first set of samples had been introduced into the rumen. On removal, the bags were washed as described previously. The residues from the rumen digestion were analyzed for DM and ash by the procedures outlined for the analysis of the initial samples described in Table 8.

Final adopted nylon bag procedure

Trial 1 Samples weighing approximately 3 g were placed in 6 x 6 cm bags. The bags were made of Saatifil nylon (Tenyl, Sao Paulo, SP, Brazil) having a pore size of 30 micrometers. Bags were constructed from a single piece of material measuring 13 x 10 cm which was folded in half. Two open edges were closed by a heat sealer. After insertion of the sample, the third open edge was closed using the same heating device. Approximately 72 cm^2 of bag surface area was exposed in the rumen, yielding a sample dry weight:bag surface area ratio of about 40 mg sample/cm^2.

The samples used have been described in Table 7. Two bags of each sample were incubated in the two fistulated steers at each of the four incubation times in two consecutive weeks (periods). Thus, eight replications for each feed or crop residue sample tested at each time of digestion (incubation) were obtained. One additional bag containing 3 g of small pieces of hard plastic (blank) was used in each incubation time in each animal to measure the entry of rumen particulate matter into the bag.

Two crossbred rumen fistulated steers fed twice daily a total of 20 kg whole plant corn silage (30% DM) and 1 kg concentrate (ground corn and soybean meal) were used in this trial.

From 10 to 15 bags were tied to a weighted ring made out

of Tygon tubing and nylon string. Another longer nylon string (40-50 cm) was used to attach the weighted ring with bags to the rumen canula. All samples were placed in the rumen at once in the morning before feeding and then taken out later in 24-h intervals; so that one Tygon tubing ring was taken from the rumen of each animal on 4 consecutive days. Thus, the incubation times were: 24, 48, 72, and 96 h.

On removal, the bags were rinsed under running water for about 20 min to remove adherent feed particles outside the bag and soluble materials from within. This was followed by successive washings in distilled water until the water squeezed from the bags was clear (3 to 5 rinses were generally required). This washing procedure was performed in order to minimize the contamination of the residues with rumen fluid, microbial matter, and small feed particles, which entered the bag from the rumen.

The bags containing samples and residues from the rumen digestion were dried at 65 C for 72 h and weighed before and after digestion, respectively. Dry matter, ash, N, and mineral analyses were conducted on the residues by the same procedures described previously for the analysis of feeds and crop residues.

Trial 2 Samples weighing approximately 3 g were placed in 8 x 9 cm bags. The bags were made of either R102 Marvelaire white dacron polyester (N. Erlanger, Blumgardt and Co., Inc.,

Broadway, NY), having an average pore size of 52 micrometers (20 to 70 micrometers) or Saatifil nylon (Tenyl, Sao Paulo, SP, Brazil). The construction of the bags was similar to that described in trial 1, except that a sewing machine was used instead of a heat sealer. The seams were glued (Instant Vinyl, Sioux City, IA) to prevent particulate matter losses through the needle holes. Approximately 144 cm^2 of bag surface were exposed in the rumen, yielding a sample dry weight:bag surface area ratio of about 20 mg/cm^2.

The samples used in this trial were described in Table 8. Except when using the nylon bags, two bags of each sample (alfalfa hay, corn stover silage, and corn cobs) were incubated in each of the three fistulated steers at each of the three incubation times on three consecutive days (periods). Thus, 18 replications for each feed or crop residue sample tested at each time of digestion (incubation) were used (dacron cloth). One nylon bag containing alfalfa hay was also incubated in each steer at each incubation time in all three consecutive periods. Thus, 9 replications were used at each time of digestion (nylon cloth). One dacron bag containing either alfalfa hay or corn stover silage which had been washed with deionized water in a shaker for 8 h (water changed every 2 h) was also incubated in each steer at each time of digestion in all three consecutive periods.

Three rumen fistulated steers (Hereford and Angus) were

used in this trial. They were fed twice daily (9 AM and 9 PM) a total of 9.1 Kg of whole plant corn silage (45% DM), 5.5 kg of corn stover silage (67% DM), and .9 kg of soybean meal.

Nine bags were tied to a weighted ring made from Tygon tubing using a nylon string. As in the previous trial, another longer nylon string was used to attach the weighted ring to the rumen canula and placed into the ventral sac of the rumen for 6, 12, or 24 h. Samples to be incubated for 24 h were introduced into the rumen first, just before the evening feeding, followed by the introduction of samples to be exposed for 12 h which were placed in the rumen the next morning before feeding, and the last samples (6-h incubation) which were introduced into the rumen 18 h after the introduction of the 24-h samples. Thus, all bags were removed at the same time, 24 h after the entry of the first set.

On removal, the bags were washed by the same procedure described for the washing of the bags in trial 1. Dry matter, N, ash, N and mineral analyses were conducted on the residues by the same procedures described previously for the analysis of initial samples shown in Table 8.

Apparent and Calculated *in vivo* True Digestibility of Silages Fed to Sheep with 4 Levels of Added Mineral Elements

Animals

Twenty-four male castrated caudectomized sheep were assigned according to their body weights to 8 treatments in a

randomized complete block design, with 32 days of adaptation and pre-experimental period and a 7-day collection period. Animals were housed in metal metabolism cages continuously.

Diets

Whole plant corn silage (31.9% DM) and elephant grass silage (24.3% DM) were used in this *in vivo* digestibility trial. The description and composition of silages are shown in Tables 9 and 10, respectively. Four levels of mineral salts were provided with both silages in a factorial combination of treatments. The mineral mixtures were designed to provide none, 1/2, 1, and 2 times the total needs of Ca and P of the sheep. Ground corn grain was used as the vehicle for feeding the urea-mineral mixtures. Increasing levels of minerals were added at the expense of the ground corn grain. Two hundred grams (air-dry basis) of the mineral mixtures were fed daily to all sheep.

The composition of the 4 mineral mixtures containing urea and ground corn grain along with their chemical analysis are presented in Tables 9 and 10, respectively.

All animals were fed silage "ad libitum" twice daily (9 AM and 4 PM). They also received 200 g of ground corn-urea mineral mixture (air-dry basis), divided into two meals which was readily consumed at the time of feeding.

Table 9. Composition of mineral mixtures containing urea and ground corn grain fed to sheep

Ingredient	Reference no.	Mineral mixtures 0	1	2	3
		----------% air-dry basis----------			
Corn grain, ground	4-21-018	90.00	87.50	85.00	77.50
Urea, 45% N	5-05-070	5.00	5.00	5.00	5.00
Dicalcium phosphate	6-01-080	-	2.50	5.00	7.50
Bone meal, steamed	6-00-399	-	-	-	5.00
Sodium chloride[a], NaCl		5.00	4.96	4.91	4.91
Magnesium oxide, MgO, CP	6-02-757	-	.02	.04	.04
Zinc oxide, ZnO, CP	6-05-554	-	.01	.03	.03
Copper sulfate, $CuSO_4$, CP		-	.01	.02	.02
Total		100.00	100.00	100.00	100.00

[a]Contained .03% $CoSO_4$ and .02% KI.

Table 10. Chemical analysis of the mineral mixtures containing urea and ground corn grain fed to sheep

Ingredient	Mineral mixtures 0	1	2	3
Dry matter	87.39	88.22	88.59	89.27
Crude protein, % DM	22.87	23.01	22.75	23.22
Ash, % DM	6.54	8.54	11.22	17.16
Calcium, % DM	.03	.71	1.51	3.43
Phosphorus, % DM	.29	.78	1.37	1.95
Magnesium, % DM	.10	.13	.16	.18
Zinc, ppm DM	23.3	147.8	271.8	264.8
Manganese, ppm DM	5.8	13.9	22.8	29.9
Copper, ppm DM	2.5	33.1	69.1	52.7

Parameters measured

Feed intake was measured, on days 21 through 28, by the difference between the amount of silage fed and that refused and weighed back. Silage DM intake was added to that of the concentrate DM in order to get the total DM intake.

Apparent digestibility and N balance were measured, on days 32 through 39, using the total collection of feces and urine methodology. Fecal collection bags were harnessed to all sheep on the thirtieth day of the digestibility trial. Urine was collected in plastic buckets containing 20 ml of HCl diluted 1:1 with water.

Samples of silage, silage orts, concentrate, feces and urine were collected daily during the 7-day collection period. They were each frozen separately at -12 C for later analysis. Then each of the 7-day respective samples from each sheep were composited for chemical determinations.

Feces and urine from the first 24 h of the collection period were discarded according to the procedure outlined by Schneider and Flatt (1975). Thus, the collection and sampling of the excretions (feces and urine) started and finished one day later than the sampling of the feeds.

All samples, except urine, were dried in an oven at 65 C for 72 h, weighed, ground finely enough to pass a 2-mm screen. All ground samples and urine were analyzed for N by the micro-Kjeldahl procedure (A.O.A.C., 1965). Gross energy was also measured in all ground samples, using an adiabatic calorimeter (Parr Instrument Co., Moline, IL). Dry matter was determined in an oven at 105 C for 24 h. Mineral elements (Ca, P, Mg, Zn, Mn, and Cu) were analyzed using the same procedures previously described for the analysis of cattle feeds and crop residues.

Assumptions for calculating true absorption of mineral elements

True absorption of minerals in silages and silage-supplemented diets was calculated assuming that the endogenous fecal excretion of Ca, Mg, Zn, and Cu were 16, 3, 0.053, and

0.0028 mg/kg liveweight per day, respectively. The endogenous fecal loss of P was assumed to vary with P intake and was given by the equation $Y = 11.6 + 10.1 X$, where Y = mg P excreted/kg liveweight per day and X = g/day of P intake (ARC, 1980).

Statistical Procedures

The data from this research were analyzed by the Statistical Analysis System (SAS) package according to the SAS user's guide manual (SAS, 1979) and by the GENSTAT procedure (GENSTAT Manual, 1977). When the analysis of variance procedure revealed significant treatment effects, the means were subjected to the multiple range test of Duncan described by Steel and Torrie (1960). The occurrence of linear and quadratic effects of the concentration of cellulase enzyme and the length of time of digestion in the rumen on the disappearance of DM, N, and mineral elements samples were tested by the GENSTAT procedure (GENSTAT Manual, 1977), and the GLM and REG procedures (SAS, 1979).

RESULTS AND DISCUSSION

Nitrogen and Mineral Composition of Cattle Feeds and Crop Residues

Preliminary usage of existing procedures with ashing modifications

The results from the two procedures employed for the determination of CP (N x 6.25), in conjunction with the P determination from the same digestion with sulfuric acid, are presented in Table 11

Both the macro-Kjeldahl (A.O.A.C., 1965) and the micro-Kjeldahl (Williams and Twine, 1967) methods gave similar CP values for the five samples tested. A t test showed no difference ($P>.05$) between the two procedures when tested either for each sample individually or for the mean of the five samples.

The P determination from the same extract of the micro-Kjeldahl digestion procedure (Williams and Twine, 1967), using an AutoAnalyzer, produced consistent results in the four trials conducted. Three of the five samples analyzed for CP were also analyzed for P later by the A.O.A.C. (1965) procedure (molybdovanadate reagent procedure), and the results were comparable as presented in Table 14. The three samples referred to are: no. 5 - corn stover, no. 11 - oat straw, and no. 13 - alfalfa hay.

Tables 12 and 13 contain the results of the comparison

Table 11. Crude protein and P content of 5 different feed samples

Feed sample	CP[a] Macro-Kjeldahl[b,c]	CP[a] Micro-Kjeldahl[d,e]	P[d,e]
	% DM		
Alfalfa hay	21.46	22.35	.29
Corn stover	3.90	3.92	.07
Dry poultry waste	19.87	20.02	2.72
Oat straw	4.16	4.26	.22
Soybean meal	47.13	47.12	.68
Mean	19.30	19.53	

[a]No statistical difference between the macro- and micro-Kjeldahl methods (P>.05).

[b]A.O.A.C. (1965) method.

[c]Mean of 4 determinations (2 observations on 2 different days).

[d]Williams and Twine (1967) procedure.

[e]Mean of 8 determinations (2 observations on 4 different days).

Table 12. Ash, Ca, K, Zn, and Cu content of feed samples ashed at 3 different temperatures and 2 lengths of time of ashing[1]

	Ashing temperature, C						
	625		550		500		
	4.0	1.5	4.0	1.5	4.0	1.5	CV[2]
	ashing time, h						
			Ash, % DM				
Alfalfa hay	7.01[a]	7.65[a]	8.57[a]	8.16[a]	8.44[a]	8.22[a]	
Corn stover	17.22[a]	15.90[a]	15.33[b]	17.48[a]	12.66[b]	16.50[a]	
Dry poultry waste	39.00[b]	39.91[b]	44.00[a]	44.08[a]	44.57[a]	44.09[a]	
Oat straw	11.17[a]	11.40[a]	11.59[a]	11.82[a]	11.79[a]	11.97[a]	
Soybean meal	6.57[a]	6.61[a]	6.93[a]	6.73[a]	6.89[a]	6.97[a]	
Mean	16.19[c]	16.29[c]	17.28[ab]	17.65[a]	16.87[bc]	17.55[ab]	4.31
			Ca, % DM				
Alfalfa hay	1.25[a]	1.28[a]	1.26[a]	1.45[a]	1.27[a]	1.46[a]	
Corn stover	.58[a]	.57[a]	.52[a]	.74[a]	.48[a]	.74[a]	
Dry poultry waste	9.24[c]	8.93[d]	9.96[b]	11.02[a]	8.85[d]	10.85[a]	
Oat straw	.17[a]	.23[a]	.27[a]	.43[a]	.35[a]	.44[a]	
Soybean meal	.25[a]	.26[a]	.26[a]	.34[a]	.24[a]	.33[a]	
Mean	2.30	2.25[c]	2.46[b]	2.80[a]	1.77[d]	2.76[a]	5.41
			K, % DM				
Alfalfa hay	1.37[b]	1.43[b]	1.49[b]	1.76[a]	1.49[b]	1.78[a]	
Corn stover	.32[b]	.43[ab]	.37[b]	.69[a]	.59[a]	.74[a]	

Dry poultry waste	1.06^{c}	1.16^{c}	1.11^{c}	1.60^{a}	1.43^{b}	1.62^{a}	
Oat straw	1.02^{e}	1.29^{d}	1.62^{c}	2.22^{a}	1.85^{b}	2.30^{a}	
Soybean meal	1.82^{b}	1.84^{b}	1.59^{c}	2.24^{a}	1.91^{b}	2.25^{a}	
Mean	1.17^{c}	1.23^{c}	1.21^{c}	1.70^{a}	1.52^{b}	1.74^{a}	5.08
			Zn, ppm DM				
Alfalfa hay	25.0^{a}	26.7^{a}	26.0^{a}	21.1^{a}	30.0^{a}	21.1^{a}	
Corn stover	17.0^{a}	20.5^{a}	28.0^{a}	25.3^{a}	25.0^{a}	26.2^{a}	
Dry poultry waste	297.0^{bc}	303.3^{abc}	317.3^{a}	279.9^{d}	306.0^{ab}	287.9^{cd}	
Oat straw	5.7^{a}	11.5^{a}	19.5^{a}	11.0^{a}	18.0^{a}	14.1^{aa}	
Soybean meal	67.0^{a}	71.7^{a}	68.0^{a}	58.0^{a}	68.5^{a}	61.3^{a}	
Mean	87.0^{b}	97.6^{a}	102.4^{a}	85.9^{b}	83.7^{b}	86.1^{b}	8.26
			Cu, ppm DM				
Alfalfa hay	15.2^{b}	16.5^{b}	34.0^{a}	12.7^{b}	13.8^{b}	11.8^{b}	
Corn stover	7.3^{a}	8.7^{a}	5.9^{a}	7.0^{a}	6.9^{a}	7.2^{a}	
Dry poultry waste	32.4^{abc}	34.7^{ab}	28.2^{a}	26.8^{c}	32.3^{bc}	25.8^{d}	
Oat straw	2.4^{a}	4.3^{a}	5.2^{a}	5.1^{a}	3.6^{a}	4.3^{a}	
Soybean meal	23.7^{a}	22.2^{a}	24.6^{a}	20.9^{a}	23.0^{a}	20.0^{a}	
Mean	16.9^{bc}	17.5^{b}	20.4^{a}	14.5^{d}	16.8^{bc}	13.8^{d}	15.64

[1]Values are the mean of 3 observations.

[2]CV = coefficient of variation in this table and all subsequent tables.

^{abcde}Means on the same row with different superscripts differ (P<.01).

Table 13. Ash, Ca, K, Mg, Zn, and Cu content of five feed samples ashed at 4 different temperatures for 1.5 h[1]

Feed sample	Ashing temperature, C				CV
	625	600	550	500	
	Ash, % DM				
Alfalfa hay	7.65[a]	7.99[a]	8.16[a]	8.22[a]	
Corn stover	15.90[a]	16.46[a]	17.48[a]	16.50[a]	
Dry poultry waste	39.91[b]	41.61[b]	44.08[a]	44.09[a]	
Oat straw	11.40[a]	11.43[a]	11.82[a]	11.97[a]	
Soybean meal	6.61[a]	6.63[a]	6.73[a]	6.97[a]	
Mean	16.29[b]	16.82[b]	17.65[a]	17.55[a]	4.79
	Ca, % DM				
Alfalfa hay	1.28[a]	1.48[a]	1.45[a]	1.46[a]	
Corn stover	0.57[a]	0.71[a]	0.74[a]	0.74[a]	
Dry poultry waste	8.93[b]	11.13[a]	11.02[a]	10.85[a]	
Oat straw	0.23[a]	0.36[a]	0.43[a]	0.44[a]	
Soybean meal	0.26[a]	0.35[a]	0.34[a]	0.33[a]	
Mean	2.25[b]	2.80[a]	2.80[a]	2.76[a]	8.85
	K, % DM				
Alfalfa hay	1.43[b]	1.79[a]	1.76[a]	1.78[a]	
Corn stover	0.43[c]	0.56[b]	0.69[a]	0.74[a]	
Dry poultry waste	1.16[b]	1.58[a]	1.60[a]	1.62[a]	
Oat straw	1.29[c]	2.13[b]	2.22[ab]	2.30[a]	
Soybean meal	1.84[b]	2.27[a]	2.24[a]	2.25[a]	
Mean	1.23[c]	1.66[b]	1.71[a]	1.74[a]	3.29

[1]Values are the mean of 3 observations.

[abc]Means on the same row with different superscripts differ ($P<.01$).

Table 13. (Continued)

Feed sample	Ashing temperature, C 625	600	550	500	CV
		Mg, % DM			
Alfalfa hay		.33[a]	.32[a]	.33[a]	
Corn stover		.43[b]	.45[a]	.46[a]	
Dry poultry waste		.48[ab]	.49[a]	.47[b]	
Oat straw		.14[b]	.17[a]	.17[a]	
Soybean meal		.32[a]	.32[a]	.32[a]	
Mean		.34[b]	.35[a]	.35[a]	2.90
		Zn, ppm DM			
Alfalfa hay	25.7[a]	22.3[a]	21.1[a]	21.1[a]	
Corn stover	20.5[a]	24.1[a]	25.3[a]	26.2[a]	
Dry poultry waste	303.3[a]	284.0[b]	279.9[b]	287.9[b]	
Oat straw	11.5[a]	10.8[a]	11.0[a]	14.1[a]	
Soybean meal	71.7[a]	64.9[b]	58.0[b]	61.3[b]	
Mean	97.6[a]	81.2[c]	85.9[b]	86.1[b]	5.63
		Cu, ppm DM			
Alfalfa hay	16.5[a]	13.2[b]	12.7[b]	11.8[b]	
Corn stover	8.7[a]	7.1[a]	7.0[a]	7.2[a]	
Dry poultry waste	34.7[a]	26.9[b]	26.8[b]	24.8[b]	
Oat straw	4.3[a]	4.7[a]	5.1[a]	4.3[a]	
Soybean meal	22.2[a]	21.4[a]	20.9[a]	20.0[a]	
Mean	17.5[a]	14.7[b]	14.5[b]	13.8[b]	7.64

of different temperatures and different length of time of ashing on the ash, Ca, Mg, K, Zn, and Cu content of the five feed samples. The analysis of variance tables along with the Duncan's multiple range test of the main effects are presented in Appendix Tables B1 and B2.

Since Heckman (1967, 1968) and Hoover (1976) had indicated that both the wet and the dry ash methods could be used for the digestion of feed samples for subsequent mineral analysis, the latter method was chosen for this work, mainly for safety reasons.

The ash content of the five feed samples, expressed as a % DM, was affected by both the temperature and the length of time of ashing. Both 550 and 500 C gave similar results while 600 and 625 C gave lower ($P<.01$) values according to the Duncan's multiple range test. This observation is in agreement with those of Heckman (1967, 1968) who recommended that 550 C be used. Ashing the samples for 1.5 h gave higher ($P<.05$) % ash than when ashing for 4 h. This result is conflicting with those of Heckman (1967, 1968) who recommended that 4 h be used. The effect of sample was also highly significant ($P<.001$). A difference in the ash content among samples was expected, since five completely different samples were used. For instance, a concentrate (soybean meal), a hay (alfalfa), two crop residues (corn stover and oat straw) and a waste resource (dry poultry waste) are known to be completely

different feeds with respect to their ash content. There was no interaction ($P>.30$) between temperature and time, indicating that these two variables were independent in the case of the ash analysis. However, the temperature x sample, time x sample, and temperature x time x sample were all significant ($P<.01$). When the Duncan test was applied for each sample individually, it showed that the interactions seemed to be due to the greater effects of temperature and length of time of ashing on corn stover and dry poultry waste than on the other three samples, even though the same tendency occurred in all samples.

The Ca content of the samples, like the ash, was affected by temperature, time and sample ($P<.001$). All four interactions (temperature x time, temperature x sample, time x sample, and temperature x time x sample) were also significant ($P<.001$). Temperature above 600 C seemed to cause loss of Ca from the samples (Table 13). Ashing the samples for 4 h gave lower Ca values than when samples were ashed for 1.5 h (Table 12). Since the temperature x time interaction was significant, it was necessary to run the Duncan test to find the best combination of temperature and time.

Ashing the samples at either 500 C or 550 C for 1.5 h gave the highest readings for Ca (Table 12). The readings at 500, 550, and 600 C were all higher than those at 625 C (Table 13) for samples ashed for 1.5 h. Some samples had a tendency

for the highest Ca reading to be at 500 C while others tended to have the highest reading at 550 C.

The cause of the interactions of both temperature and time of ashing with sample seemed to be due to the significant decrease ($P<.01$) observed in the Ca concentration of dry poultry waste (DPW) when either the temperature or the time of ashing increased. This decrease in Ca concentration was not significant ($P>.01$) for the other samples.

The Mg content of samples, like the ash and Ca, was also affected by the temperature of ashing. This effect, although significant ($P<.001$), was small. The interaction of temperature and sample was also significant ($P<.001$). The highest Mg content of DPW was observed when the samples were ashed at 550 C while similar values of Mg were observed in both corn stover and oat straw when the temperature of ashing was increased from 500 to 550 C. No change in Mg concentration was observed in alfalfa hay and soybean meal when the temperature of ashing increased from 500 to 600 C (Table 13).

The K content of samples was affected by temperature, time and sample. Ashing the samples for 1.5 h gave higher K values than when ashing them for 4 h ($P<.001$). Ashing the samples at 500 C also gave higher K values than ashing them at either 550 or 625 C ($P<.05$); however, a significant temperature x time interaction was observed. Increasing the ashing temperature from 500 to 550 C caused a decrease ($P<.01$) in the

% K of most samples when they were ashed for 4 h but not for 1.5 h (Table 12). Ashing samples at 600 C for 1.5 h gave lower ($P<.01$) K concentration than ashing them at either 550 or 500 C for 1.5 h (Table 13). A significant ($P<.001$) time x sample interaction was observed. An increase of only 14% in the K content of alfalfa hay was observed compared to an increase of 48% in corn stover sample when the ashing time was decreased from 4 to 1.5 h. The other three samples had increases in K content within this range when the length of time of ashing was changed from 4 to 1.5 h.

A large coefficient of variation (CV) was observed when analyzing the feedstuffs for Zn and Cu content as compared to the analysis of similar feedstuffs for the content of macroelements (Ca, Mg, K) (Tables 12 and 13). A higher ($P<.01$) Zn content of DPW and a higher ($P<.01$) Cu content of DPW and alfalfa hay were observed when ashing these samples at 625 C than when ashing at lower temperatures (Tables 12 and 13). This effect is the opposite of that observed when analyzing similar feedstuffs for the content of macroelements.

Final adopted procedures

Because of the large CV observed in the Zn and Cu determinations of feeds (Tables 12 and 13), the sample size was increased from 1 to 3 g and the solubilized ash transferred to 25 ml volumetric flasks instead of the 100 ml flasks used in the previous preliminary studies.

The best combination of temperature of ashing and length of time of ashing for the analysis of mineral elements in feeds was 550 C and 1.5 h (Tables 12 and 13). Thus, the feed samples and crop residues described in Tables 7 and 8 were analyzed for CP, ADF, IVDMD, ash, Ca, P, Mg, K, Zn, Mn and Cu, using the adapted procedures of Heckman (1967, 1968) according to the results of the previous preliminary studies.

The results of these analyses are presented in Tables 14 and 15. The 8 forage feeds and 11 crop residues described in Table 7 could be divided into two groups according to their CP content: one containing less than 6% CP and the other more than this value. The lower CP group (<6%) would be composed of 10 crop residues and crop residue plant parts, and a tropical grass silage (elephant grass silage). All of these feeds (lower CP group) do not meet the requirements for CP of a nonlactating beef cow, which is 5.9% of the DM (NRC, 1976). This low CP group also had a high average ADF content (47.8%) and a low digestibility of DM in nylon bags (43.4%) as shown in Table 24. All of these low CP feeds, except soybean pods, are unable to meet the energy requirements (Table 1) of a nonlactating beef cow, which is 52% TDN (NRC, 1976); i.e., assuming normal levels of feed intake.

The higher CP group (>6%) is composed of 7 forage

Table 14. Chemical composition of samples used in subsequent nylon bag and cellulase digestibility studies

Roughage	CP	ADF	Ash	IVDMD	Ca	P	Mg	K	Zn	Mn	Cu
	% DM								ppm DM		
Soybean stover	3.8	56.6	3.8	36.9	.89	.06	.56	.71	10.5	20.0	16.8
Soybean stalks	3.2	64.4	4.5	28.9	.82	.08	.38	.53	8.1	15.9	3.6
Soybean leaves	10.1	39.2	30.3	39.0	2.93	.12	.58	.38	31.7	189.4	8.9
Soybean pods	5.2	35.1	9.1	63.0	1.30	.09	.71	2.28	31.0	23.6	5.1
Corn stover	3.6	48.3	11.7	45.8	.66	.06	.42	.93	10.8	50.5	5.5
Corn stover	4.0	40.6	6.2	53.9	.46	.08	.32	.98	15.1	34.3	5.3
Corn stover silage	4.2	41.6	10.5	42.5	.56	.10	.34	.96	20.4	41.7	5.4
Corn husks	2.4	44.6	3.2	60.0	.17	.04	.11	.65	10.8	36.6	3.2
Cornstalks	4.0	48.0	6.4	41.8	.22	.10	.11	2.32	23.5	32.2	4.8
Corn leaves	5.3	46.2	9.0	44.6	.54	.10	.25	.57	10.1	79.6	3.6
Oat straw	4.5	47.8	10.6	39.4	.36	.20	.14	2.59	7.4	20.1	2.6
Alfalfa hay	14.1	40.0	8.2	56.6	1.41	.27	.32	2.12	26.7	34.6	23.5
Alfalfa hay	21.0	31.7	7.9	59.0	1.46	.27	.31	2.10	21.3	27.4	15.7
Alfalfa, 2nd cut	18.8	32.9	9.3	58.2	1.20	.30	.22	3.08	21.1	28.6	14.6
Reed canarygrass	21.0	30.7	12.0	44.5	.41	.29	.28	3.2	40.1	63.7	15.7
Smooth brome-grass	16.5	32.0	12.5	56.8	.77	.32	.23	2.11	13.6	63.1	7.0
Tall fescue	15.3	33.7	11.1	56.3	.50	.30	.33	3.28	20.2	54.9	16.0
Whole plant corn silage	6.2	36.0	4.6	59.2	.13	.15	.16	1.05	19.3	59.3	3.6
Elephant grass silage	3.8	52.6	9.0	47.2	.33	.16	.22	1.64	13.3	211.8	2.3

Table 15. Chemical composition of samples used in subsequent nylon bag digestibility studies

Roughage	CP	Ash	Ca	P	Mg	Zn
	% DM					ppm DM
Alfalfa hay	23.4	9.6	1.48	.27	.36	33.5
Corn cobs	2.0	1.7	.02	.04	.04	19.3
Corn stover silage	5.3	9.2	.43	.11	.21	15.4
Whole plant corn silage	8.7	5.7	.38	.23	.25	20.9

feeds and a crop residue (soybean leaves). Whole plant corn silage (grown in Brazil) would barely meet the CP needs for a nonlactating beef cow and would not meet the maintenance requirement for CP of a 300-kg growing calf. The other forage feeds analyzed contained more than 14% CP; therefore, they do meet the requirements for CP of any class of Beef or Dairy animal listed in Table 1, assuming expected levels of DM intake under ad libitum feeding conditions. This higher CP group had a lower average ADF content (34.5%) than the low protein group and a higher average digestibility of DM in nylon bags (60.4%). Over one-half the energy fed in the total dairy production system is supplied by rations containing 60 to 63% TDN (Klopfenstein and Owen, 1981). Also, both the lactating and the dry Beef cow need less than 60% TDN in their diets (NRC, 1976). Therefore, high quality roughages can meet

this TDN requirement.

The CP values of the 2 forage feeds and the 2 crop residues shown in Table 15 are in close agreement with the data of Table 14, which have been discussed previously.

The contents of CP and ADF of the crop residues studied in this work are comparable to those of Elhag (1976), Cunha (1974), and Vetter (1975).

The ash content of the forage feeds and the crop residues shown in Tables 14 and 15 varied from 1.7% in corn cobs to 30.3% in soybean leaves. The content of ash of all samples, except that of soybean leaves, was within the expected range of ash for the specific sample (Vetter, 1973; Cunha, 1974; NRC and Canada Dept. Agric., 1971; McDowell et al., 1974). The latter reference (Latin American Tables of Feed Composition) cited ash content of soybean leaves lower than that found in this work. There is no apparent reason for the high ash content of this crop residue plant part, because the plant parts were separated by hand after the harvest of the whole plant. On the other hand, it is known that the ash fraction of plants contains varying amount of silica. Wolff, 1871, as cited by Thorpe and Whiteley (1937) observed that silica composed 35-40% of the ash in grasses, but only 2-4% in legumes. Thus, the ash content of crop residues could be affected by plant species (Gupta and Pradhan, 1975) and/or by soil contamination depending on the harvesting machinery utilized.

For example, corn stalklage and milo stover harvested using Hesston equipment showed a higher ash content than the corn and sorghum plant parts which composed these stovers (Vetter, 1973).

It is doubtful whether the knowledge of the composition of soybean leaves is important in the practical feeding of crop residues. Baled soybean stover was either devoid of leaves or contained very small amounts (Vetter, 1973).

The Ca content of forage feeds and crop residues was high. Only corn husks, cornstalks, whole plant corn silage (grown in Brazil) shown in Table 14, and corn cobs shown in Table 15, would not meet the .35% Ca needs of most classes of Beef and Dairy animals presented in Table 1, assuming expected levels of feed intake. The content of Ca of the crop residues and forage feeds analyzed was in close agreement with the data of Vetter (1973), and within the range of calcium listed by the NRC (1976), Latin American Tables of Feed Composition (McDowell et al., 1974), and the Atlas of Nutritional Data on US and Canadian Feeds (NRC and Canada Dept. Agric., 1971).

Like the CP content, these crop residues and forage feeds could also be divided into two groups, according to their P content: one containing less than .2% P and the other more than .2% P. The low P group contained all the crop residues and crop residue plant parts, plus whole plant corn silage (grown in Brazil) and elephant grass silage (Table 14). The

higher P group was composed of the forage feeds, with the exception of the silages grown in Brazil. The P content of the corn silage grown in Iowa was higher than that grown in Brazil. The other feed and crop residues shown in Table 15 contained similar amounts of P as those shown in Table 14. The feeds and crop residues from the low P group would meet the requirements for P of a nonlactating beef cow (NRC, 1976). In this respect, these data are in agreement with those of Vetter (1973), even though the values reported here are somewhat lower than those reported by the previous author.

All 19 samples of forage feeds and crop residues shown in Table 14 were higher than .1% Mg; and only corn cobs (Table 15) was lower than this value, which is adequate to meet the requirements for Mg of all classes of Beef cattle shown in Table 1, with the exception of the lactating cow (NRC, 1976). Of the 23 samples analyzed, only 5 (corn husks, cornstalks, whole plant corn silage, corn cobs and oat straw) were below the .2% Mg requirement for a Dairy cow (NRC, 1978). The content of Mg of these samples was within the range of that reported by other workers (Vetter, 1973; NRC, 1976).

The K content of all crop residue and forage feed samples analyzed were high. Only 3 crop residue plant parts (soybean stalks, soybean leaves, and corn leaves) were lower than .6% K, while only 1 (soybean leaves) was lower than .5% K. Ward (1966) reviewed the K metabolism literature and concluded that

the dietary requirement of ruminants for this element is no more than .5% DM. The NRC (1976) recommended from .6 to .8% while the NRC (1978) recommended .8% K of the total DM. The K content of the forage feeds and crop residues reported in this work is in agreement with those reported elsewhere (NRC, 1976; Vetter, 1973).

About one-half of the forage feeds and crop residues analyzed had a Zn concentration lower than the 20 ppm needed by most classes of Beef and Dairy animals listed in Table 1; while about two-thirds (2/3) of the same samples were lower than the 10 ppm requirement for Cu for the Dairy animals (NRC, 1978). These samples low in Zn and Cu content included most crop residues, smooth bromegrass, whole plant corn silage (grown in Brazil) and elephant grass silage. The content of Zn and Cu of crop residues reported in this work are lower than those reported by Vetter (1973). However, the Zn and Cu content of the alfalfa feed samples were similar to those values reported by NRC (1978).

Only one of the 19 forage feeds and crop residues (Table 13) analyzed (soybean stalks) would not meet the requirements for Mn (20 ppm) as stated by the ARC (1980) or NRC (1976). However, more than half of the samples analyzed would not meet the requirements for Mn (40 ppm) listed by the NRC (1978). The samples within the range of 20 to 40 ppm included the alfalfa hays, most crop residues and crop residue plant parts,

except leaves. Thus, depending on the safety margins needed for this element (Mn) in practical feeding programs (Miller, 1983), the content of Mn in more than half of the forage feeds and crop residues analyzed in this work could be considered either marginal or deficient.

The crop residues, in general, were low in CP, digestibility of DM (energy), P, Zn, Cu, and perhaps Mn, depending on the requirements for these elements for livestock. The protein and mineral composition of crop residues, among other factors, is influenced by the harvesting process, plant nutrition and maturity, the % of leaves recovered in the harvested residue, and environmental factors (Vetter, 1975).

Modification of the IVDMD

The results from the two trials concerning the modification of the washing step of the IVDMD procedure (Tilley and Terry, 1963) are shown in Table 16. The analysis of variance table and the Duncan's multiple range test used to compare the means in trial 1 are shown in Appendix Table B3.

In trial 1, the use of a second digestion phase (24 h) with pepsin-HCl increased ($P<.05$) the average *in vitro* disappearance of DM of five feed samples from 61 to 66%, and decreased ($P<.05$) the ash content of the residue of digestion from 25 to 14% of DM. Changing the washing step of the procedure, from the standard wash recommended by the authors of

Table 16. *In vitro* DM disappearance (IVDMD) and ash content of the residues of five feeds and crop residues determined by either the one-stage or the two-stage procedure, followed by either standard or "exhaustive" washing of the residue

	One-stage digestion			Two-stage digestion				
	Standard		"Exhaus."	Standard		"Exhaustive"		
Sample	Tr. 1	Tr. 2	Tr. 1	Tr. 1	Tr. 2	Tr. 1	Tr. 2	CV
			IVDMD[1], %					
Alfalfa hay	61.0^{bc}	58.6^{c}	63.9^{ab}	65.2^{a}	64.6^{a}	67.2^{a}	54.5^{d}	
Corn stover	59.9^{a}	55.1^{b}	59.6^{a}	53.7^{b}	53.8^{b}	53.8^{b}	53.2^{b}	
Dry poultry waste	42.7^{b}	37.5^{c}	39.5^{c}	63.1^{a}	64.2^{a}	64.7^{a}	63.6^{a}	
Oat straw	56.4^{b}	49.8^{d}	53.4^{bc}	64.3^{a}	50.5^{cd}	51.3^{cd}	50.6^{cd}	
Soybean meal	84.6^{cd}	82.6^{d}	88.7^{ab}	90.7^{a}	86.2^{bc}	88.9^{ab}	85.0^{cd}	
Mean	61.0^{h}	56.7	61.0^{g}	67.4^{e}	63.8^{g}	65.2^{f}	61.4^{h}	2.4
			Ash in the residue[2], % DM					
Alfalfa hay	13.6^{a}	12.5^{a}	11.3^{a}	$.7^{b}$	1.2^{b}	$.8^{b}$	1.2^{b}	
Corn stover	27.2^{a}	24.9^{ab}	24.4^{b}	19.4^{c}	20.1^{c}	19.6^{c}	20.5^{c}	
Dry poultry waste	70.1^{a}	64.3^{b}	65.6^{b}	38.6^{d}	36.5^{d}	41.9^{c}	38.5^{d}	
Oat straw	11.2^{a}	11.4^{a}	10.1^{ab}	8.3^{b}	9.0^{ab}	8.3^{b}	9.7^{ab}	
Soybean meal	5.8^{b}	11.0^{a}	3.7^{bc}	4.0^{bc}	4.8^{bc}	4.1^{bc}	2.2^{c}	
Mean	25.6^{e}	24.8^{e}	25.1^{e}	14.2^{f}	14.3^{f}	14.9^{f}	14.4^{f}	4.8

[1]Values are the mean of 3 observations. Tr. = trial.

[2]Values are the mean of 2 observations.

^{abcd}Means on the same row with different superscripts differ ($P<.01$).

^{efghi}Means on the same row with different superscripts differ ($P<.05$).

the IVDMD procedure to an "exhaustive" wash, caused a small but significant decrease ($P<.05$) in IVDMD and no change in the ash content of the residue, when these two washing procedures were compared.

The purpose of such an "exhaustive" washing of the residue was to guard against contamination of this residue from the high level of minerals added from artificial saliva by the in vitro digestion system. It was also aimed at reducing the possible contamination of the residue of digestion with undigested microbial matter. However, either the "exhaustive" washing system proposed was not able to remove the contamination or the artificial saliva did not contaminate the residue, as judged by the ash content of the residue, which remained unchanged.

Among other drawbacks of the proposed modification of the IVDMD, one could mention the increased length of time needed to wash the residue, and also the increased difficulty of filtering the residue from "exhaustive" washing when it was compared with the standard washing step of the procedure. The latter problem was especially true when the "exhaustive" washing followed a one-stage digestion (rumen fermentation only). Thus, in trial 2, this treatment (one-stage digestion + "exhaustive" washing) was omitted.

Even though the mean IVDMD of the 5 feed samples in trial 2 was smaller than in trial 1, somewhat similar results were

obtained when the treatments were compared.

There are some problems associated with the use of the rumen fluid *in vitro* system to predict availability of nutrients. According to Osbourn and Terry (1977), the variation in the proportion of the apparently digested OM arising from cell walls severely limits the usefulness of the single stage *in vitro* procedures. A second stage using a solvent or enzyme to solubilize protein is invariably required in addition to an estimate of the cell wall degradation. This second stage of digestion also aids in solubilizing the ash remaining in the residue. For instance, the digestibility of the ash *in vivo* was 47% on the average for six tropical grasses compared with 71% *in vitro* (McLeod and Minson, 1974). Troelsen (1970) attempted to determine the energy value of the OM digested *in vitro*, by the difference between the energy in the feed sample and that in the residue from a two-stage *in vitro* technique. The *in vitro* assay (Troelsen, 1970) did not directly reproduce the *in vivo* digestible energy levels over the entire quality range of the 102 hays tested.

Sample size also could be a problem. The original technique (Tilley and Terry, 1963) uses .5 g of feed sample. Some laboratories use an even smaller sample size. Under these conditions, the weight of the undigested residue will be small; therefore, it would be difficult to determine its mineral content. Increasing the sample size ten times (from .3 to 3 g) does not seem feasible.

Solubilization with Enzymes

Measurement of activity of cellulase enzyme

The activity of the cellulase enzyme derived from Trichoderma viride was tested with increasing concentrations of the enzyme to establish the maximum solubilization of the DM of feeds that could be achieved. No account was taken of the cellulase activity quoted by the suppliers because it referred to activity for carboxymethyl cellulose and, according to Jones and Hayward (1975), this bore no discernible relationship to true cellulolytic activity. The results of this test are shown in Table 17. The T. viride cellulase had low activity as measured by the DM solubilized during a 24 h incubation. Increasing concentrations of cellulase caused small increases in the amount of DM disappearance from corn stover and solka floc (cellulose), but considerably larger amounts of soybean meal were digested under these same conditions. The results are in agreement with the data of Roughan and Holland (1977) who reported that cell walls of whole, dried plant material were either not attacked by the cellulase or were attacked very slowly. Jones and Hayward (1973) reported somewhat better results, showing a 42% solubilization of DM of cocksfoot (60% in vitro DM digestibility), after 24 h incubation with 6.25 g/l of cellulase. Jones and Hayward (1973) also showed small increases in the amount of DM solubilized from cocksfoot when the enzyme concentration was increased from 6.25 to 25

Table 17. Effect of increasing enzyme concentration on amount of DM solubilized after 24 h incubation at 40 C with cellulase (Trichoderma viride)[a,b,c]

	Enzyme/test tube, mg					
	Buffer only	30	60	120	240	480
	DM solubilized, %					
Corn stover	14.0	16.1	17.8	19.2	21.1	22.4
Soybean meal	32.9	53.9	59.9	65.1	70.4	75.3
Cellulose (Solka floc)	3.9	6.9	7.7	7.8	8.6	10.8

[a]Values are the mean of 2 observations.

[b]Enzyme dissolved in 30 ml of citrate-phosphate buffer (pH 4.8).

[c]300-mg sample.

g/l. Their observation agrees with the results reported in this work.

Measurement of solubilization of DM and ash by four different enzymes

The results from the solubilization of DM and ash from alfalfa hay, corn stover, dry poultry waste, oat straw, and soybean meal, by protease WT, amylase WT, cellulase WT (A. niger), cellulase (T. viride) and two buffers, are presented in Table 18. The analysis of variance table together with the comparison of the treatment means by both the orthogonal contrasts and the Duncan's multiple range test are shown in Appendix Table B4. The mean ash content in the residue from solubilization with either buffers or buffer plus enzymes is presented in Appendix Table A1.

The protease and amylase enzymes showed similar effects. They solubilized considerably larger amount of soybean meal than did the pH 7.0 buffer alone. However, the effect of these two enzymes on the other four feed samples was small.

On the average for the five feed samples tested, T. viride cellulase solubilized more ($P<.05$) dry matter than A. niger cellulase. Although statistically significant, the difference between the DM solubilized by the two cellulases was small, only 2.5 digestibility units. This result is in disagreement with that of Jones and Hayward (1975) and McQueen and Van Soest (1975) who reported that T. viride cellulase

Table 18. Solubilization of DM and ash of five feed samples by four different enzymes incubated at 40 C for 48 h[1,2,3]

Feed samples	Buffer pH 7.0			Buffer pH 4.8			CV
	Buffer only	Protease WT	Amylase WT	Buffer only	Cellulase WT	Cellulase (*T. viride*)	
	DM solubilized, %						
Alfalfa hay	45.2	48.0	45.7	32.2	43.6	47.0	
Corn stover	15.2	16.5	18.5	14.4	16.5	20.2	
Dry poultry waste	21.1	25.3	26.2	40.1	42.4	43.0	
Oat straw	22.0	23.7	24.9	18.9	21.4	23.0	
Soybean meal	37.4	74.9	72.0	30.6	56.1	59.3	
Mean	28.2^{d}	37.7^{b}	37.5^{b}	27.3^{e}	36.0^{c}	38.5^{a}	1.2
	Ash solubilized, % DM						
Alfalfa hay	58.5	62.3	73.1	77.6	87.5	86.4	
Corn stover	19.7	30.8	29.5	39.7	36.8	34.2	
Dry poultry waste	12.4	10.5	15.5	54.1	49.2	51.3	
Oat straw	52.5	52.1	52.0	51.1	50.0	48.4	
Soybean meal	74.3	84.5	87.9	82.0	89.9	90.2	
Mean	43.5^{f}	48.0^{e}	51.6^{d}	60.9^{c}	62.7^{a}	62.1^{b}	0.6

[1]Values are the mean of 2 observations.

[2]300 mg sample/test tube.

[3]180 mg enzyme dissolved in 30 ml of its respective buffer/test tube.

[abcdef]Means on the same row with different superscripts differ ($P<.05$).

was much more active than A. niger cellulase.

The cellulases and the pH 4.8 buffer solubilized more ($P<.05$) ash than did the neutral enzymes and the pH 7.0 buffer. These higher values of the ash solubility seemed to be due more to the acidity of the buffer itself than to the enzymes themselves.

All enzymes would seem to have underestimated the DM digestibility of all five feed samples, when these values are compared with those found in the feed composition tables (NRC, 1976; NRC and Canada Dept. Agric., 1971; McDowell et al., 1974).

Effect of pretreatment on the solubilization by cellulase enzymes

The effects of seven different pretreatments (during 24 h at room temperature) on the DM solubilized by cellulase WT, tested in trial 1, are presented in Table 19. The effects of .1 N HCl and .2% pepsin dissolved in .1 N HCl (24 h at 40 C), followed by increasing levels of T. viride cellulase (48 h at 40 C) on the solubilization of DM, CP, and ash, tested in trial 2, are presented in Table 20. The content of CP, ash, and mineral elements in the residue from the factorial combination of all treatments of trial 2 is presented in Appendix Tables A2 and A3. The analysis of variance tables and the Duncan's multiple range tests of the effects of the pretreatments on the solubilization of samples by cellulase enzymes are shown in Appendix Tables B5 and B6.

Table 19. Effect of seven pretreatments on the DM solubilized by cellulase WT and buffer at 40 C for 24 h, and the ash content of the residue of digestion, trial 1[1,2,3]

	Alfalfa hay		Corn stover		
	Buffer only	Cellulase WT	Buffer only	Cellulase WT	CV
	DM solubilized, %				
None	31.6^{d}	42.5^{b}	14.1^{c}	13.3^{c}	
H_2O	32.6^{c}	42.3^{b}	16.3^{ab}	16.0^{b}	
pH 4.8 buffer	32.9^{c}	42.8^{b}	15.6^{b}	16.6^{ab}	
.001 N HCl	33.3^{c}	42.2^{b}	16.3^{ab}	16.5^{ab}	
.01 N HCl	33.3^{c}	43.7^{a}	16.8^{a}	16.6^{ab}	
.1 N HCl	34.2^{b}	43.7^{a}	15.6^{b}	16.8^{ab}	
2 N HCl	37.6^{a}	42.4^{b}	15.7^{b}	17.3^{a}	
Mean	33.6	42.8	15.8	16.2	1.1
	Ash in the residue, % DM				
None	3.0^{a}	2.2^{a}	13.7^{a}	13.4^{c}	
H_2O	3.2^{a}	2.5^{a}	13.7^{a}	14.0^{bc}	
pH 4.8 buffer	3.0^{a}	2.0^{a}	12.7^{b}	13.8^{c}	
.001 N HCl	3.1^{a}	2.2^{a}	10.2^{c}	13.7^{c}	
.01 N HCl	3.0^{a}	2.0^{a}	10.6^{c}	15.0^{a}	
.1 N HCl	2.4^{a}	1.7^{a}	12.0^{b}	14.1^{bc}	
2N HCl	.9^{b}	.7^{b}	12.6^{b}	14.8^{ab}	
Mean	2.7	1.9	12.2	14.1	3.7

[1]Values are the mean of 2 observations.

[2]1.0 g sample.

[3]100 ml of buffer or 100 ml of 6.25 g/l of enzyme dissolved in buffer.

[abcd]Means on the same column within each analysis, with different superscripts differ ($P<.01$).

Table 20. Effect of HCl and pepsin-HCl pretreatments (24 h at 40 C) followed by incubation with increasing levels of T. viride cellulase (48 h at 40 C), on the solubilization of DM, CP, and ash of alfalfa hay and corn stover, trial 2

Pretreatment	Cellulase, g/l				CV
	Buffer only	6.25	12.50	25.00	
	DM solubilized[1], %				
Alfalfa hay					
.1 N HCl	40.0^{c}	65.5^{c}	66.8^{a}	63.4^{b}	
.2% pepsin	52.4^{c}	71.1^{a}	71.0^{a}	67.1^{b}	
Corn stover					
.1 N HCl	18.4^{c}	42.3^{a}	42.1^{a}	36.1^{b}	
.2% pepsin	18.6^{c}	42.5^{a}	42.5^{a}	36.4^{b}	
Mean	32.4^{f}	55.4^{e}	55.6^{d}	50.8^{e}	2.1
	CP solubilized[2], %				
Alfalfa hay					
.1 N HCl	39.4^{c}	60.5^{b}	66.2^{a}	66.3^{a}	
.2% pepsin	80.6^{a}	82.4^{a}	83.5^{a}	84.1^{a}	
C rn stover					
.1 N HCl	32.5^{b}	51.9^{a}	52.8^{a}	53.8^{a}	
.2% pepsin	52.8^{b}	58.5^{a}	61.5^{a}	61.4^{a}	
Mean	51.3^{f}	63.3^{e}	66.0^{d}	66.4^{d}	2.0
	Ash solubilized[2], %				
Alfalfa hay					
.1 N HCl	90.5^{b}	95.6^{a}	97.3^{a}	97.0^{a}	
.2% pepsin	90.7^{b}	96.4^{a}	96.8^{a}	97.2^{a}	
Corn stover					
.1 N HCl	45.3^{c}	54.4^{a}	48.2^{b}	47.9^{b}	
.2% pepsin	45.6^{c}	52.4^{a}	47.5^{c}	49.3^{b}	
Mean	68.0^{f}	74.7^{d}	72.4^{e}	72.9^{e}	1.0

[1]Values are the mean of 2 trials with 2 observations each.

[2]Values are the mean of 2 observations.

[abc]Means on the same row with different superscripts differ ($P<.01$).

[def]Means on the same row with different superscripts differ ($P<.05$).

Some pretreatments at room temperature (Table 19) caused a small but significant ($P<.01$) increase in the digestibility of DM and a decrease in the ash content of the residue remaining after incubation with enzymes. However, a much larger increase in the digestibility of DM was obtained when the pretreatment was at 40 C (Table 20). In trial 2 (hot treatment), the digestibility of DM of alfalfa hay and corn stover approached the expected levels of digestibility of these feeds (feed composition table values) when these samples were pretreated with .2% pepsin-HCl for 24 h at 40 C, before the incubation with cellulase. The maximum solubilization of the DM, CP, and ash was obtained when samples were pretreated with .2% pepsin-HCl, followed by incubation with 6.25 g/l of cellulase enzyme dissolved in citrate-phosphate buffer.

Pepsin treatment of samples before incubation with cellulase caused an increase ($P<.01$) in DM and CP solubilized from alfalfa hay and CP solubilized from corn stover. It had no effect on the ash solubilized from both samples when this treatment was compared with the acid treatment.

The solubilization of DM, CP, and ash from similar feed samples were compared with the disappearance of DM, CP, and ash from nylon bags suspended in the rumen, and will be included in the discussion of the nylon bag technique procedure which follows later.

The concentration of mineral elements in the residue from

the two-stage pepsin-cellulase digestion study is shown in Appendix Table A3. A low concentration of all mineral elements was found in the residue from all treatments; thus, the solubility of each individual mineral element was not calculated. This unrealistically high solubility of mineral elements was probably caused by the hydrochloric acid (HCl) used in the pretreatment period. For instance, Ward et al. (1979) reported that 1 N HCl solution extracted 96.8% of the total calcium in alfalfa samples.

The improvement of the solubilization of feed DM by pepsin-HCl before the incubation with cellulase enzyme found in this work is in agreement with results reported by several authors. Jones and Hayward (1975) observed an increase in the DM solubilized by pretreating the samples with .2% pepsin dissolved in .1 N HCl (24 h, 40 C). Allison and Borzucki (1978) found a further increase in the cellulase solubilization of the DM by increasing the acid concentration of the Jones and Hayward (1975) procedure from .1 N to .125 N and by raising the pretreatment temperature from 40 to 50 C. Roughan and Holland (1977), using a neutral-detergent extraction (90 min, 110 C) before the treatment with cellulase, showed an increase in the DM disappearance, while Kellner and Kirchgessner (1977) found a similar increase in DM disappearance, using a 3-stage procedure composed of refluxing samples at 100 C for 30 min with 2 N HCl, which was followed by two 24 h incubations with cellulase and pepsin, in this order.

Measurement of activity of a Brazilian cellulase enzyme and solubilization of feeds and crop residues with pepsin-HCl as pretreatment

The results from the test of the activity of the cellulase enzyme from Biobras (Montes Claros, MG, Brazil), measured by the solubilization of DM, CP, and ash of smooth bromegrass, are shown in Figure 1 and in Appendix Table A4.

The analysis of variance and the Duncan's multiple range test indicated that increasing levels of cellulase digested increasing amounts of DM, CP, and ash (Appendix Table A4). The maximum solubilization of the DM, CP, and ash was obtained with cellulase concentration of 6 g/l ($P<.05$), shown in Appendix Table A4. This result is in agreement with the recommendation of Jones and Hayward (1973, 1975) that 6.25 g/l of cellulase be used.

The regression analysis showed that the quadratic model fit the data. The curves shown in Figure 1 were the result of fitting the following regression equations to the data presented in Appendix Table A4.

Dry matter solubilized:

$Y = 40.01 + 3.61\ X - 0.20\ X^2$ ($r^2=0.95$, $P<.0001$, rsd=1.46);

Crude protein solubilized:

$Y = 70.40 + 3.16\ X - 0.18\ X^2$ ($r^2=0.99$, $P<.0001$, rsd=0.59);

Ash solubilized:

$Y = 71.11 + 1.20\ X - 0.06\ X^2$ ($r^2=0.95$, $P<.0001$, rsd=0.57);

where Y is the % solubility of either the DM, CP, or ash and

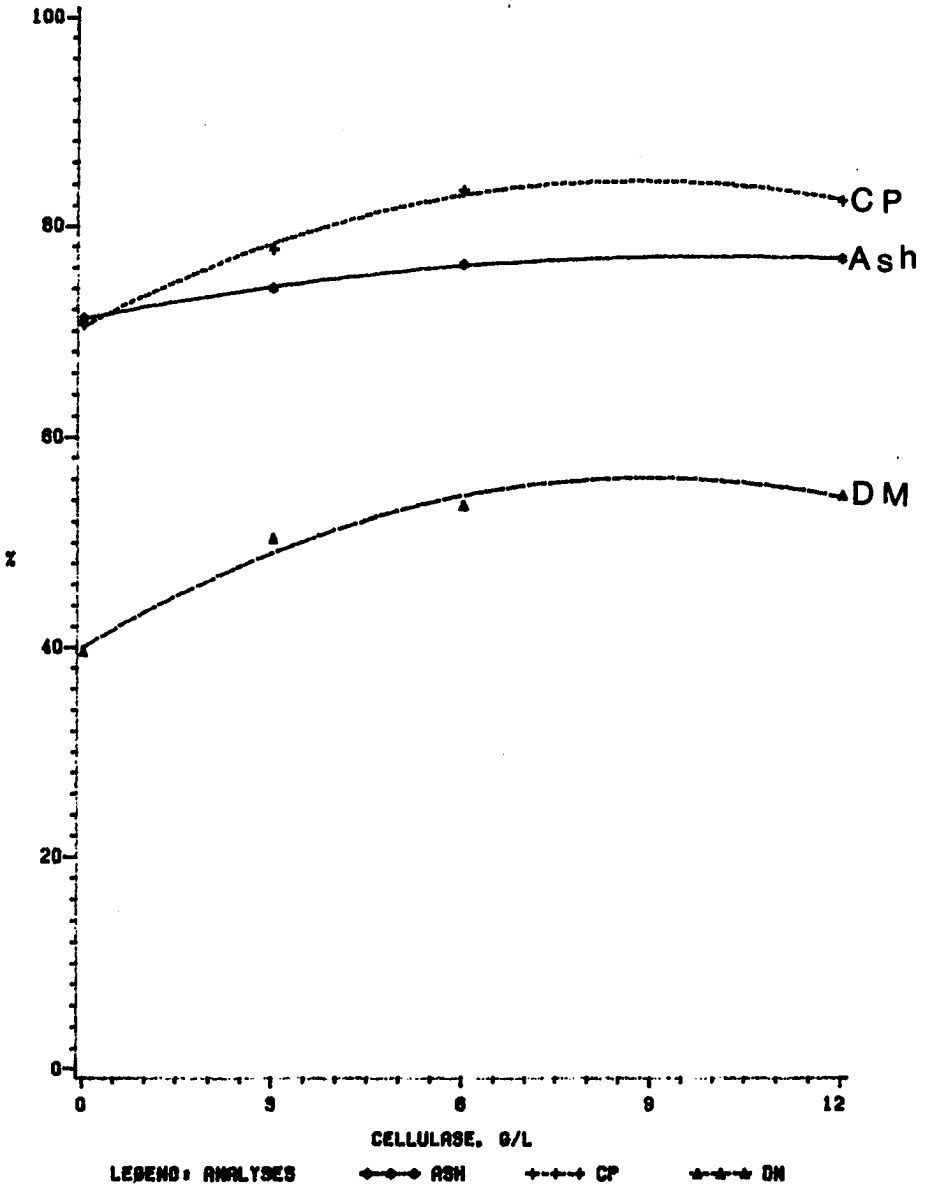

Figure 1. Solubilization of DM, CP, and ash of smooth bromegrass by pepsin-HCl (24 h), followed by incubation with increasing levels of cellulase enzyme (48 h) at 40 C

X is g/l of cellulase enzyme dissolved in citrate-phosphate buffer (pH 4.8) and rsd is the residual standard deviation.

The results from the solubilization of DM, CP, OM, and ash of the feeds and crop residues described in Table 7, with pepsin-HCl followed by cellulase enzyme, are presented in Table 24, together with the results from the disappearance of the same fractions of these feed samples in nylon bags suspended in the rumen.

The concentrations of CP, ash and mineral elements in the residues of digestion with pepsin-HCl followed by incubation with cellulase enzymes are shown in Appendix Table A5.

The linear regression equations comparing the solubilization of samples by the two-stage pepsin-cellulase technique and the two-stage nylon bag-pepsin technique are presented together with the results from the nylon bag technique procedure (Appendix Table B10).

The level of mineral elements in the residue from the solubilization of the 17 feed and crop residue samples by the pepsin-cellulase technique, shown in Appendix Table A5, were considered low; thus, their solubilities were found to be unrealistically high. This was probably caused by the HCl added to the pepsin used in the pretreatment period. Nevertheless, the pretreatment seems necessary in order to achieve the digestibility of DM and OM similar to that found *in vivo* or with other laboratory techniques (nylon bag, IVDMD). Since

the concentrations of mineral elements in the residue from all samples were found to be low, the insoluble ash determined by the pepsin-cellulase procedure seemed to give a rough measure of the acid-insoluble ash (silica) of these feeds and crop residues.

Nylon Bag Technique

Preliminary test

The digestibility *in situ* of alfalfa hay and corn stover described in Table 6 is shown in Appendix Table A7.

The 66.8% disappearance of DM of alfalfa hay observed in this preliminary test *in situ* (nylon bag technique) is similar to the value of 65.8% found by Van Keuren and Heinemann (1962), and somewhat higher than the values reported by Neathery (1969) and lower than the 70% digestibility value of the Australian alfalfa hay reported by Playne et al. (1978a,b). The 52% disappearance of DM of corn stover in nylon bags is in agreement with the digestibility of the DM of this crop residue reported by Vetter (1973), Colenbrander et al. (1973), Vetter and Boehlje (1978). However, it is much higher than the 18-24% disappearance of DM of cornstalks from nylon bags, reported by Neathery (1969).

The digestibility of the DM *in situ* of these two samples in nylon bags is also in close agreement with the IVDMD of these samples, shown in Table 16.

The 90% disappearance of the P from the nylon bags containing alfalfa hay samples is in agreement with the isotope studies of Lofgreen and Kleiber (1954) who found that 94% of the P consumed from alfalfa was absorbed by sheep. Playne et al. (1978b) reported a release of about 82% of P from alfalfa samples suspended in nylon bags in the rumen.

The small difference in release of phosphorus measured by Playne's technique and the one reported in this work (82 vs 90) could be explained by the difference in these two *in situ* procedures. The former technique was a one-stage procedure (rumen digestion) while the latter was a two-stage procedure (rumen digestion + pepsin-HCl).

Digestion *in situ* time assay

The results of digestion of alfalfa hay and corn stover in nylon bags suspended in the rumen for 12, 24, 48, and 72 h, followed by a 24-h incubation with pepsin-HCl are shown in Table 21 and Appendix Table A8. The analysis of variance tables and the linear and quadratic regression analysis of these data are shown in Appendix Tables B7 and B8, respectively.

The results of 3 more trials, using another nylon cloth (TETKCO, Elmsford, NY) of the same pore size (20 micrometers), are shown in Table 22 and Appendix Table A9. The analysis of variance tables of these data are presented in Appendix Table B9.

Table 21. Disappearance of DM, OM, CP, ash, and P from nylon bags containing alfalfa hay and corn stover samples in nylon bags suspended in the rumen for 12, 24, 48, and 72 h, followed by a 24-h incubation in pepsin-HCl[1]

	Rumen digestion time, h				
	12	24	48	72	CV
Disappearance of DM, %					
Alfalfa hay	29.6^{c}	53.2^{b}	66.8^{a}	66.3^{a}	
Corn stover	6.0^{d}	16.5^{c}	41.6^{b}	46.5^{a}	
Mean	17.8^{h}	34.9^{g}	54.2^{f}	56.4^{e}	2.9
Disappearance of OM, %					
Alfalfa hay	26.2^{c}	49.8^{b}	64.3^{a}	64.0^{a}	
Corn stover	4.7^{d}	15.9^{c}	41.4^{b}	48.1^{a}	
Mean	15.5^{h}	32.9^{g}	52.8^{f}	56.0^{e}	3.1
Disappearance of CP, %					
Alfalfa hay	19.5^{c}	68.7^{b}	83.5^{a}	81.9^{a}	
Corn stover	-49.4^{b}	-50.3^{b}	33.3^{a}	34.3^{a}	
Mean	-15.0^{g}	9.2^{f}	58.4^{e}	58.1^{e}	4.8
Disappearance of ash, %					
Alfalfa hay	69.4^{c}	92.9^{b}	96.0^{a}	93.6^{b}	
Corn stover	15.9^{d}	21.0^{c}	43.2^{a}	34.8^{b}	
Mean	42.7^{h}	57.0^{g}	69.6^{e}	64.2^{f}	1.2
Disappearance of P, %					
Alfalfa hay	63.5^{c}	70.5^{b}	86.5^{a}	88.8^{a}	
Corn stover	-61.1^{c}	-102.7^{b}	33.3^{a}	15.9^{b}	
Mean	1.2^{g}	-16.1^{h}	59.9^{e}	52.4^{f}	3.9

[1]Values are the mean of 2 observations.

^{abcd}Means on the same row with different superscripts differ ($P<.01$).

^{efgh}Means on the same row with different superscripts differ ($P<.05$).

Table 22. Disappearance of DM, OM, CP, ash and P from nylon bags containing alfalfa hay and corn stover samples in nylon bags suspended in the rumen for 24, 48 and 72 h, followed by a 24-h incubation in pepsin-HCl, in trials 1, 2, and 3

	Alfalfa hay			Corn stover			
	24	48	72	24	48	72[1]	CV
Sample	Rumen digestion time, h						
Disappearance of DM, %							
Trial 1[2]	54.2^{b}	65.9^{a}	68.8^{a}	24.0^{d}	31.6^{d}	45.0^{c}	4.5
Trial 2[3]	62.2^{b}	66.6^{a}	68.4^{a}	25.7^{d}	38.5^{c}	43.9^{c}	4.3
Trial 3[3]	60.0^{c}	67.4^{b}	70.0^{a}	30.2^{f}	45.8^{e}	54.5^{d}	1.4
Mean	59.7^{b}	66.7^{a}	69.2^{a}	26.8^{a}	39.6^{d}	50.5^{c}	5.7
Disappearance of OM, %							
Trial 1[2]	50.7^{b}	63.5^{a}	66.4^{a}	22.7^{d}	31.5^{c}	45.4^{b}	4.5
Trial 2[3]	59.1^{b}	63.9^{a}	65.8^{a}	24.9^{e}	38.0^{d}	44.2^{c}	4.7
Trial 3[3]	56.7^{c}	65.0^{b}	67.7^{a}	28.7^{e}	45.5^{d}	55.5^{c}	1.5
Mean	56.4^{b}	64.2^{a}	66.8^{a}	25.7^{e}	39.4^{d}	51.2^{c}	6.0
Disappearance of CP, %							
Trial 1[2]	84.4^{a}	88.5^{a}	90.7^{a}	31.3^{c}	11.9^{d}	45.6^{b}	3.1
Trial 2[3]	87.4^{b}	89.2^{a}	90.7^{a}	44.7^{d}	47.5^{d}	45.6^{c}	1.7
Trial 3[3]	87.5^{b}	91.1^{a}	91.0^{a}	54.8^{d}	37.7^{e}	56.5^{c}	0.9
Mean	86.8^{a}	89.7^{a}	90.8^{a}	45.1^{b}	33.1^{c}	54.1^{b}	8.7

Disappearance of ash, %							
Trial 1[2]	92.7[a]	93.0[a]	94.9[a]	29.8[c]	32.6[c]	42.2[b]	2.4
Trial 2[3]	96.0[a]	96.6[a]	96.9[a]	31.9[c]	42.0[b]	42.4[b]	1.8
Trial 3[3]	96.5[a]	93.9[b]	94.4[b]	41.5[d]	47.6[c]	46.8[c]	0.7
Mean	95.4[a]	94.7[a]	95.3[a]	34.6[c]	41.7[b]	45.0[b]	4.4
Disappearance of P, %							
Trial 1[2]	86.1[a]	90.4[a]	93.3[a]	28.7[b]	-9.0[c]	-1.3[c]	4.0
Trial 2[3]	90.8[b]	92.6[a]	94.5[a]	46.6[c]	41.4[d]	49.2[c]	1.5
Trial 3[3]	93.5[b]	95.0[a]	94.7	62.9[c]	40.7[d]	31.0[e]	0.8
Mean	90.4[a]	93.0[a]	94.2[a]	48.1[b]	26.7[c]	28.2[c]	14.6

[1]Only one observation in trials 1 and 2.

[2]Values are the mean of 2 observations, unless otherwise specified.

[3]Values are the mean of 3 observations, unless otherwise specified.

[abcdef]Means in the same row with different superscripts differ ($P<.01$).

The regression analysis revealed that the quadratic model fit the observed data better than did the linear model. The quadratic equations shown in Appendix Table B8 gave predictions of digestibility close to the actual values for both samples, except for the digestibility of CP and P of corn stover. High negative digestibility of these two components of the DM was observed at 12 and 24 h of rumen digestion time.

Microbial matter may contain more than 10% N (Hungate, 1966), of which 85% is true protein (Church, 1976). Thus, a portion (15% of N) is nonprotein nitrogenous compounds, which include the nucleic acids. Therefore, the negative digestibility of N and P (faster rate of entry than exit) might support the hypothesis that the residues of the two-stage procedure (rumen digestion _in situ_ + pepsin-HCl) were contaminated with microbial matter, possibly attached to the cell walls of undigested fibrous residue. Mathers and Aitchison (1981) studied the contamination of feed samples digested _in situ_ for 24 h. They reported that one-fifth (1/5) of the residual N, in the nylon bags containing lucerne samples, was of microbial origin. Their technique did not have the second digestion stage with pepsin-HCl, which was part of the procedure used in the previous trials to describe the feed samples in this work. Mathers and Aitchison (1981) also mentioned that the resulting contamination of feed residues by microbial matter would lead to the greatest underestimation of protein

degradation where feeds are low in protein content, but potentially high in microbial degradability.

The extent of digestion of alfalfa hay in nylon bags was greater than that of corn stover ($P<.001$). Differences in digestibility of DM among feeds in nylon bags have been demonstrated by several authors (Playne et al., 1978a,b; Neathery, 1969; Van Keuren and Heinemann, 1962).

As the length of time of digestion in the rumen increased, the disappearance of DM, OM, CP, ash, and P increased ($P<.001$). Playne et al. (1978a,b), Neathery (1969), and Van Keuren and Heinemann (1962), all have shown similar results for the disappearance of DM while Playne et al. (1978b) showed similar results for the release of CP and P from nylon bags during digestion of feed samples in the rumen.

A significant sample x digestion time interaction observed in this work is in agreement with the work of Neathery (1969), and Playne et al. (1978a), who reported interactions between sample and length of digestion time in the rumen. The Duncan's multiple range test gave evidence that the disappearance of alfalfa hay from nylon bags reached a maximum after 48 h in the rumen while the disappearance of corn stover continued to increase with time of digestion in the rumen up to the longest time (72 h) studied.

The difference in digestibility among trials, that was observed (Table 22), was attributed mainly to the difference

in the feeding regimen of the fistulated animal as it influenced the environment of the experimental samples. In trial 1, elephant grass silage was fed to the fistulated animal, while in trials 2 and 3, whole plant corn silage was fed. Van Keuren and Heinemann (1962) reported differences among digestibility trials, while Orskov et al. (1981) recommended that rumen conditions should be such that substrates for maximum microbial fermentation are not limiting the degradation of the feed sample.

Another problem which could have contributed to the observed difference among trials, was the pore size (mean aperture diameter) of the nylon cloth. It seems that a small pore size might limit gas release from the bags. As a result, gas accumulation may occur, which causes the bags to float in the rumen. Uden et al. (1974) reported that lack of gas release from 20 to 35 micrometer pore size bags limited digestibility of feed samples contained in nylon bags. In order to prevent this problem and yet maintain the particulate matter loss through the mesh at a low level, Orskov et al. (1981) recommended that the pore size of the synthetic fiber material be within the range of 30 to 100 micrometers.

Like in the cellulase enzyme digestion technique described previously, the level of mineral elements in the residue of digestion (Appendix Tables A8 and A9), in the nylon bag technique, was remarkably low. The values of P were the

exceptions. They varied from day to day, as can be seen in the Appendix tables. One of the possible explanations for this variation could be the washing procedure employed, even though all bags were washed until the rinsing water was clear in all trials.

The summary of the disappearance of DM, OM, CP, and ash of alfalfa hay and corn stover samples determined by either the nylon bag (in situ) or the cellulase digestion technique is presented in Table 23. There was good agreement between the two laboratory techniques. The largest discrepancy between these techniques was related to the release of CP from feed samples during digestion. The nylon bag technique resulted in a higher disappearance of CP of alfalfa hay and lower disappearance of CP of corn stover than did the cellulase procedure. One of the possible reasons for this inconsistency between the techniques is the contamination of residues of digestion with microbial matter in the nylon bag procedure. Mathers and Aitchison (1981) reported that samples of lower protein content showed higher contaminations with microbial matter, expressed as a % of the total N in the bag.

Solubilization of feeds and crop residues in situ and in cellulase enzymes

The average digestibility of DM (51.1 vs 42.4) and of OM (50.2 vs 40.3) of 15 feeds and crop residues when measured by the nylon bag-pepsin technique were 9 and 10 digestibility

Table 23. Summary of the disappearance of DM, OM, CP, and ash of alfalfa hay and corn stover samples determined by the pepsin-cellulase and the nylon bag-pepsin techniques

	Alfalfa hay		Corn stover	
Sample technique	Pepsin-cellulase[a]	Nylon bag-pepsin[b]	Pepsin-cellulase[a]	Nylon bag-pepsin[b]
Disappearance of:				
DM, %	71.14	67.41	42.54	45.75
OM, %	69.84	65.01	41.06	45.50
CP, %	82.43	91.11	58.47	37.68
Ash, %	96.45	93.93	52.42	47.61

[a]24 h in .2% pepsin-HCl and 48 h in 6.25 g/l of cellulase in citrate-phosphate buffer (pH 4.8).

[b]48 h in nylon bags suspended in the rumen and 24 h in .2% pepsin-HCl.

units higher ($P<.05$) than when determined by the pepsin-cellulase technique, respectively (Table 24). However, the average digestibility of CP tended to be higher ($P<.10$) when analyzed by the pepsin-cellulase than when determined by the nylon bag-pepsin technique (69.1 vs 64.7). There was no difference between techniques when measuring the solubilization of the ash (67.4 vs 68.0) of these samples.

The regression coefficients of the linear regression analysis used to compare these techniques are shown in Appendix

Table 24. Digestibility of DM (DDM), OM (DOM), CP (DCP), and ash (DAsh) of 15 feed and crop residue samples determined by two laboratory techniques[1]

Sample	Nylon bags-pepsin				Pepsin-cellulase			
	DDM	DOM	DCP	DAsh	DDM	DOM	DCP	DAsh
Soybean stover	38.7^{g}	37.1^{g}	58.8^{c}	80.3^{b}	35.5^{e}	33.8^{e}	67.2^{e}	78.1^{c}
Soybean stalks	29.6^{i}	27.7^{i}	46.3^{d}	69.6^{cd}	28.6^{f}	26.2^{f}	60.0^{f}	78.3^{c}
Soybean leaves	58.0^{d}	63.7^{a}	62.5^{c}	45.0^{g}	59.4^{b}	59.8^{a}	61.6^{f}	58.6^{f}
Soybean pods	62.4^{bc}	59.1^{bc}	79.8^{ab}	95.6^{a}	58.5^{b}	55.0^{b}	76.9^{c}	94.4^{a}
Corn stover	51.1^{4}	51.0^{d}	60.8^{c}	52.3^{f}	34.0^{e}	32.3^{e}	67.0^{e}	61.0^{ef}
Corn stover silage	42.8^{f}	41.5^{f}	46.1^{d}	53.8^{f}	34.5^{e}	31.8^{e}	56.2^{g}	57.6^{f}
Corn husks	44.6^{f}	44.9^{ef}	45.8^{d}	65.1^{cde}	27.8^{fg}	27.0^{f}	54.8^{g}	50.1^{g}
Corn stalks	34.5^{h}	32.1^{h}	42.1^{d}	69.7^{cd}	25.0^{f}	21.9^{g}	60.5^{f}	70.0^{d}
Corn leaves	43.3^{f}	43.5^{f}	43.8^{d}	41.3^{g}	32.7^{e}	32.4^{e}	56.5^{g}	36.2^{h}
Oat straw	50.9^{e}	49.5^{de}	62.1^{c}	62.2^{de}	35.2^{e}	32.5^{e}	73.2^{d}	58.4^{f}
Alfalfa hay	59.5^{bcd}	57.2^{c}	85.8^{a}	85.4^{b}	52.3^{c}	49.4^{c}	79.2^{bc}	85.6^{b}
Alfalfa, 2nd cut	66.5^{a}	63.5^{a}	89.1^{a}	96.0^{a}	62.6^{a}	59.2^{a}	84.7^{a}	96.6^{a}
Reed canarygrass	63.0^{b}	62.4^{ab}	87.4^{a}	68.0^{cd}	48.2^{d}	45.6^{d}	81.3^{b}	68.6^{d}
Smooth bromegrass	59.3^{cd}	59.7^{bc}	76.8^{b}	56.3^{ef}	54.7^{c}	54.0^{b}	79.8^{bc}	59.3^{f}
Tall fescue	51.8^{bc}	60.7^{ab}	84.3^{ab}	70.9^{c}	46.1^{d}	43.1^{d}	78.1^{bc}	66.9^{de}
Mean	51.1	50.2	64.7	67.4	42.4	40.3	69.1	68.0
CV	3.8	3.7	8.1	5.5	4.8	5.1	3.3	6.1

[1]Values are the means of 2 trials with 2 observations each.

^{abcdefghi}Means on the same column with different superscripts differ ($P<.05$).

Table B10. Even though the regressions comparing the DM, OM, CP, and ash solubilized by the pepsin-cellulase (X) and that solubilized by the nylon bag-pepsin (Y) were all significant ($P<.001$), the CV and RSD were high for all four equations. This suggests that the cellulase technique would require further study if it were to be used to predict the *in situ* digestibility of forages and crop residues.

A high RSD and CV were also reported by Adegbola and Paladines (1977) when regressing the % DM digestibility in pepsin-cellulase with the *in vitro* DM disappearance of 24 tropical grasses and legumes.

Neathery (1969) reported considerably lower DM disappearance from nylon bags of the higher fiber roughages (crop residues) when the results were compared with published data obtained by conventional digestion trials. One of the possible explanations for the difference between the digestibility values reported in this work and that reported by Neathery (1969) could be the large sample size in relation to the bag surface area used in the latter work.

The analysis of variance tables of the data shown in Table 24 are presented in Appendix Table B11. There were differences ($P<.001$) among samples in digestibility of DM, OM, CP, and ash. The Duncan's test ranked the 15 feed and crop residue samples into 4-6 distinct ($P<.05$) groups, as shown in Table 24.

Forage feeds and crop residues ranked by the previous test were divided into 4 digestibility classes. Sometimes, feeds from two distinct groups (according to the Duncan's test) were combined into one digestibility class. Figures 2 and 3 show the relative frequency of each class of digestibility of DM (DDM), OM(DOM), CP (DCP), and ash (DAsh), according to either the nylon bag-pepsin or the pepsin-cellulase techniques.

All classes (relative frequency) of digestibility of DM, and OM, except the lowest (<40%) were lower when the samples were analyzed by the pepsin-cellulase technique than when analyzed by the nylon bag technique (Figure 2). The overall distribution (histograms) of the digestibility of DM and OM seemed to have been displaced to the right; that is, to the lower end of digestibility, when the feed samples were analyzed by the pepsin-cellulase technique.

The relative frequency of the four classes of digestibility of the ash (Figure 3) presented in the form of histograms showed a similar pattern of distribution when the nylon bag and the cellulase techniques were compared, despite the fact there were some differences in the relative frequency of the individual classes of digestibility of DM and OM. Thus, the residual ash does not seem to be associated with the residual fiber (undigested cell walls) when analyzed by these two techniques. A possible explanation for the similarity in the disappearance of the ash in both techniques is the presence

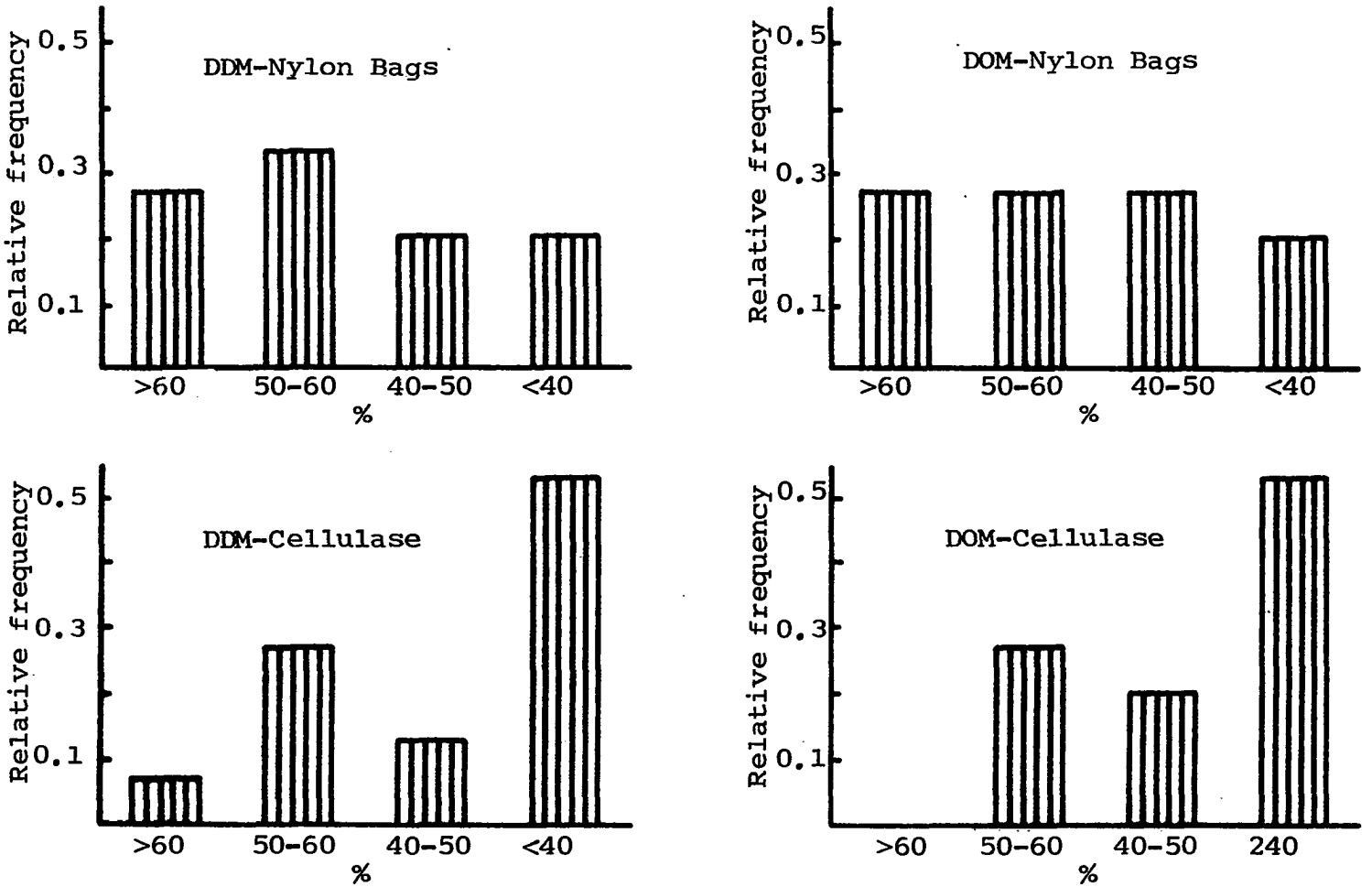

Figure 2. Relative frequency classes of digestibility of DM (DDM), and OM (DOM) of 15 feed and crop residue samples digested either by the nylon bag-pepsin or by the pepsin-cellulase techniques

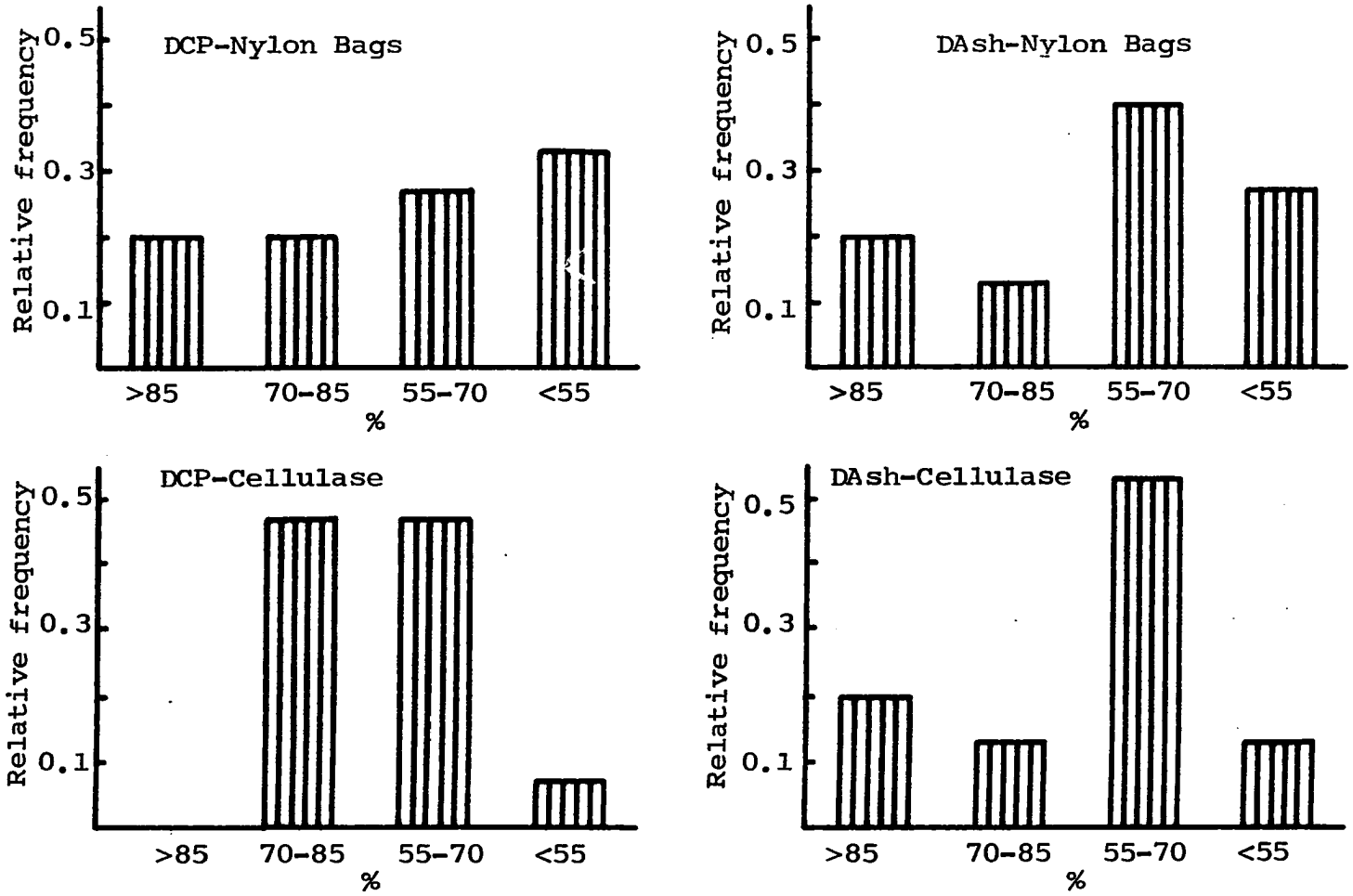

Figure 3. Relative frequency classes of digestibility of CP (DCP) and ash (DAsh) of 15 feed and crop residue samples digested either by the nylon bag-pepsin or by the pepsin-cellulase techniques

of .1 N HCl used to dissolve the pepsin enzyme, which was used in the first stage of one procedure and in the second stage of the other. Ward et al. (1979) showed that 1 N HCl could extract 96.8% of the Ca of ground alfalfa samples.

The histograms of the digestibility of CP show that the solubility of this fraction in pepsin-cellulase seemed to have been displaced to the center of the distribution from both ends when compared with the nylon bag-pepsin technique. The highest and the lowest digestibility classes were either absent or had only one observation in the pepsin-cellulase technique, while the middle two classes had a relative frequency higher in this technique than in the nylon bag-pepsin technique. The higher proportion of samples in the lower digestibility class (<55%) in the nylon bag technique could be explained by contamination of the residue of digestion with microbial matter. The absence of any feed samples in the highest (>85%) digestibility class in the pepsin-cellulase procedure is probably related to the low digestibility of the OM shown in Figure 2, when the feeds were analyzed by the pepsin-cellulase technique.

Pepsin treatment

The effect of a second stage of digestion with pepsin-HCl was studied by measuring the disappearance of DM, OM, CP, ash, Ca, P, Mg, K, Zn, Mn, and Cu from nylon bags containing either one of the 4 feeds: alfalfa hay and corn stover

(Table 25) and whole plant corn silage and elephant grass silage (Table 26). The effect of increasing the length of time of digestion in the rumen on the disappearance of the same components from samples contained in nylon bags also was studied (Table 25).

The content of CP and mineral elements in the residue remaining after incubation with or without pepsin-HCl as a second digestion stage are presented in Appendix Tables A10 and A11. The analysis of variance tables and the Duncan's multiple range test are shown in Appendix Tables B12 and B13.

A second digestion stage with pepsin-HCl, after the fermentation of feed samples contained in nylon bags suspended in the rumen, had no effect ($P>.05$) on the digestibility of OM of alfalfa hay (Table 25) and silages (Table 26). It had an inconsistent effect on the digestibility of OM of corn stover (Table 25). However, it caused a marked increase ($P<.01$) in the digestibility of CP of alfalfa hay and corn stover (Table 25) and elephant grass silage (Table 26). Pepsin-HCl treatment also caused a marked increase ($P<.001$) on the disappearance of all mineral elements studied.

As the digestion time in the rumen increased, the disappearance of OM, CP, and almost all mineral elements studied increased ($P<.01$). The disappearance of the OM of alfalfa hay reached a maximum after 48 h of digestion in the rumen, while the disappearance of OM of corn stover continued to increase

Table 25. Effect of pepsin-HCl and time of digestion in the rumen on the digestibility of DM (DDM), OM (DOM), CP (DCP), ash (DAsh), Ca (DCa), P (DP), Mg (DMg), K (DK), Zn (DZn), Mn (DMn), and Cu (DCu) of alfalfa hay and corn stover in nylon bags[1,2]

	24 h		48 h		72 h	
Sample	+	-	+	-	+	-
			Alfalfa hay			
DDM	60.0^{c}	57.0^{cd}	67.4^{ab}	66.1^{b}	70.0^{a}	67.1^{ab}
DOM	56.7^{b}	54.4^{b}	65.0^{a}	64.4^{a}	67.7^{a}	65.6^{a}
DCP	87.5^{ab}	73.3^{c}	91.1^{a}	83.8^{b}	91.0^{a}	84.8^{b}
DAsh	96.5^{a}	85.1^{b}	93.9^{a}	84.3^{b}	94.4^{a}	83.8^{b}
DCa	98.7^{ab}	72.2^{e}	98.4^{ab}	76.2^{d}	97.8^{b}	75.1^{d}
DP	93.5^{ab}	88.8^{b}	95.0^{a}	91.6^{ab}	94.7^{a}	90.5^{ab}
DMg	99.8^{a}	84.4^{c}	99.9^{a}	92.0^{b}	99.9^{a}	92.3^{b}
DK	99.9^{a}	99.2^{b}	100.0^{a}	99.5^{b}	100.0^{a}	99.5^{b}
DZn	100.0^{a}	-97.1^{e}	100.0^{a}	47.0^{b}	100.0^{a}	28.2^{c}
DMn	100.0^{a}	-30.7^{e}	100.0^{a}	30.4^{c}	100.0^{a}	33.2^{bc}
DCu	83.4^{b}	71.8^{e}	89.4^{a}	81.6^{b}	91.2^{a}	78.8^{c}

[1]Values are the mean of 3 observations.

[2]+ or - pepsin.

[abcdefghi]Means on the same row with different superscripts differ ($P<.01$).

24 h		48 h		72 h		
+	-	+	-	+	-	CV
		Corn stover				
30.2^{g}	26.1^{g}	45.8^{f}	49.5^{e}	54.5^{d}	55.0^{d}	2.8
28.7^{c}	25.0^{f}	45.5^{d}	49.6^{c}	55.5^{b}	56.4^{b}	3.0
54.8^{d}	24.3^{g}	37.7^{e}	23.9^{g}	56.5^{d}	33.7^{f}	2.8
41.5^{d}	34.8^{e}	47.6^{c}	48.4^{c}	46.8^{c}	44.0^{d}	1.9
99.5^{ab}	68.4^{f}	99.7^{a}	76.7^{cd}	99.4^{ab}	78.4^{c}	0.9
62.9^{c}	42.3^{d}	40.7^{d}	3.7^{f}	31.0^{e}	-5.5^{g}	3.4
99.0		99.3		99.3		0.5
98.8^{c}	91.2^{f}	99.2^{b}	93.3^{e}	99.2^{b}	94.6^{d}	0.2
100.0^{a}	-390.4^{f}	100.0^{a}	-96.4^{e}	100.0^{a}	-80.8^{d}	740.7
95.5^{a}	21.3^{d}	97.5^{a}	37.3^{b}	97.5^{a}	27.2^{cd}	4.9
74.6^{d}	46.3^{i}	68.4^{f}	64.2^{g}	70.2^{ef}	59.9^{f}	1.4

Table 26. Effect of pepsin-HCl on the digestibility of DM (DDM), OM (DOM), CP (DCP), ash (DAsh), Ca (DCa), P (DP), Mg (DMg), K (DK), Zn (DZn), Mn (DMn), and Cu (DCu) of whole plant corn silage and elephant grass silage in nylon bags for 48 h[1,2]

	Corn silage		Elephant grass silage		
	+	-	+	-	CV
DDM	47.9[a]	50.2[a]	32.1[b]	31.6[b]	10.5
SOM	47.5[a]	50.2[a]	30.3[b]	30.3[b]	10.7
DCP	74.2[a]	67.6[a]	46.8[b]	30.1[c]	7.3
DAsh	56.1[a]	50.2[a]	50.2[a]	44.9[a]	9.8
DCa	100.0[a]	31.2[b]	99.8[a]	41.3[b]	7.9
DP	90.5[a]	82.8[b]	85.2[a]	72.8[c]	2.7
DMg	99.8[a]	79.8[c]	99.9[a]	89.7[b]	1.4
DK	99.8[a]	99.0[b]	99.9[a]	98.8[b]	0.2
DZn	91.0[a]	-15.2[ab]	91.3[a]	-91.9[b]	242.9
DMn	100.0[a]	87.1[b]	100.0[a]	85.4[b]	1.8
DCu	40.9[a]	27.9[a]	12.1[a]	-43.6[b]	149.7

[1]Values are the mean of two trials with 2 observations each.

[2]+ or - pepsin.

[abc]Means on the same row with different superscripts differ ($P<.01$).

up to the longest time (72 h) studied. The significant sample x time of digestion interaction observed in this trial is in agreement with the results from all previous trials reported in this work. It is also in agreement with the results from the work of Neathery (1969) and Playne et al. (1978a).

When the pepsin-HCl digestion phase was used, the disappearance of almost all mineral elements from bags reached values above 90%, even after the shortest digestion time (24 h) studied. These values are in contrast with the relatively low coefficient of absorption of these mineral elements reported in the literature.

Even though the residual Ca in the bags, expressed as a % of DM, was 3 times higher in alfalfa hay than in corn stover, as shown in Appendix Table A12 (1.0% vs 0.3% Ca), the release of this element from alfalfa hay and corn stover contained in nylon bags suspended in the rumen was about 76% for both feed samples. The disappearance value of Ca from alfalfa hay (76%) is in agreement with the conclusion of Ward et al. (1979) that the Ca in alfalfa is only 50 to 75% as available to cattle as that from inorganic sources. Furthermore, these authors also reported that the chemical equivalence of oxalate present in alfalfa averaged 24% of that of Ca in the hay samples studied. Playne et al. (1978b) reported that 65% of the Ca of alfalfa hay disappeared from the nylon bags after 48 h of

digestion in the rumen.

The release of Ca from samples contained in nylon bags during digestion in the rumen (Table 25) was higher than the disappearance of Ca from these samples in water (Table 27). This seems to indicate that a portion of the Ca from alfalfa hay and corn stover could only be released after the digestion or probably partial digestion of the cell walls. On the other hand, the release of N and P from corn stover samples in water was higher than after digestion in nylon bags in the rumen. This may indicate either contamination of the residual matter with microbial matter or a lack of a concentration gradient between the undigested residues and the rumen fluid surrounding the bags.

The disappearance of P and Mg from alfalfa hay in water was slightly lower than the amount digested from alfalfa during the first 24 h in the rumen.

The high disappearance values of Mg were in agreement with the release of this mineral element from 4 tropical hays reported by Playne et al. (1978b). However, the release of Mg from feed in nylon bags was much higher than the coefficient of absorption of dietary Mg (.26) reported by the ARC (1980). It is important to realize that removal of a nutrient from digesting feed in the rumen, and even its solubility in water may not be related to net availability. Removal of a nutrient from digesting feed is only indicative that it may be poten-

tially available to the rumen microflora or to the animal for their use. Fitt et al. (1974) found that the rumen microbial cell-walls show an affinity for Mg and Ca; moreover, Fitt and Hutton (1974) reported that increasing concentrations of K reduces the Mg binding capacity of the microbial cell-walls. This affinity of the rumen microbial cell-walls for Mg may account for the large difference between the net availability of Mg (.26) and the solubilization of Mg from plant material reported in this work (92%) and in the work of Playne et al. (1978b) (79%).

Beveridge and Murray (1980) reported that the carboxyl groups of the glutamic acid residues of the cell wall protein of Bacillus subtilis (gram positive bacterium) could bind several metals. The cell walls of these bacteria can contain large amount (50% of dry weight) of teichoic acid. Teichoic acid is composed mainly of glycerol-phosphate with some ester-linked D-alanine and D-glucose.

If the undigested residues were contaminated with microbial matter as suggested previously, this binding of metals by the bacterial cell walls could explain the negative digestibility of some of the trace elements studied.

Calcium from whole plant corn silage and elephant grass silage (grown in Brazil, Table 26) was released during digestion in nylon bags in the rumen to a lesser extent than the release of Ca from alfalfa hay and corn stover produced in

Iowa. This could be related to the lower initial concentration of this element in both silages than in alfalfa hay and corn stover from Iowa. Playne et al. (1978a) reported that the proportions of elements removed from nylon bags during digestion in the rumen were positively related to the initial concentration of the elements in feeds.

The release of Ca from whole plant corn silage and elephant grass silage from nylon bags suspended in the rumen is in disagreement with the true absorption of Ca in the in vivo digestibility study of these two silages (Table 38). While the nylon bag technique showed no difference ($P<.01$) between the Ca disappearance from whole plant corn silage and elephant grass silage (31.2 vs 41.3%), the calculated true absorption of Ca in vivo by sheep was significantly different ($P<.05$). The values of the in vivo absorption of Ca were 65.5% and 28.6% for whole plant corn silage and elephant grass silage respectively. The difference from the in vivo calculated true absorption of Ca in corn silage and elephant grass silage could be related to the significant difference ($P<.05$) in the intake of Ca from these two silages with no Ca supplementation (mineral mixture no. 0), shown in Table 36. Braithwaite (1978a), Scott and McLean (1981), and Abdel-Hafeez et al. (1982) have reported that sheep and cattle absorb Ca from their gut according to need. These authors also showed that the animal can alter the efficiency of absorption of Ca to meet a change in requirement.

The release of P during digestion of whole plant corn silage and elephant grass silage in nylon bags in the rumen was similar to the in vivo calculated true absorption of this element (Table 38).

The release of Mg of whole plant corn silage and elephant grass silage from nylon bags during digestion in the rumen were 80 and 90%, respectively. These values are higher than the in vivo calculated true absorption of this element, 67.6 and 34.9% for whole plant corn silage and elephant grass silage, respectively.

Losses of particulate matter and water-soluble nutrients from bags

Movement of fine particles through pores of the cloth occurred in all 6 trials conducted (Tables 27, 28, 29, and Figures 4 and 5). Losses of particulate matter averaged 7.8% of the original DM present in the bag in trials 1 and 2, and 2.2% in trial 3. A nylon cloth with 20 micrometer pore size was used in these trials (Table 27) while a nylon cloth with 30 micron pore size was used in trial 4 (Table 28). The loss of particulate DM in this trial (trial 4) varied from 2.8% to 14.2% of the original DM of the 15 feed and crop residue samples analyzed. When a 52 micron dacron polyester cloth was used in trial 5 (Table 29), the losses of particulate matter varied from 2.6% to 11.7%.

Playne et al. (1978a) reported that the losses of

Table 27. Losses of particulate DM and water-soluble DM, OM, CP, ash, Ca, P, and Mg from nylon bags (20 micron pore size) containing alfalfa hay and corn stover samples, during extraction with water, expressed as % of total element initially present

	Alfalfa hay			Corn stover			
	Tr. 1[1,2]	Tr. 2[1,2]	Tr. 3[3,4]	Tr. 1[1,2]	Tr. 2[1,2]	Tr. 3[3,4]	CV
Loss of particulate DM	6.9[a]	8.0[a]	2.5[b]	9.2[a]	7.0[a]	1.9[b]	21.1
Water-soluble DM	28.9[b]	35.4[a]	26.2[b]	17.5[c]	16.9[c]	10.5[d]	6.4
Water-soluble OM	24.8[b]	32.0[a]	22.3[b]	11.8[c]	13.1[c]	6.0[d]	7.5
Water-soluble ash	73.8[a]	73.3[a]	67.9[a]	52.4[b]	45.3[c]	3917[d]	3.1
Water-soluble CP	35.6[ab]	28.4[b]	29.6[b]	49.3[a]	44.8[ab]	27.5[b]	14.7
Water-soluble Ca	48.3[b]	48.6[b]	37.3[c]	56.6[a]	56.7[a]	37.6[c]	5.3
Water-soluble P	86.8[a]	86.8[a]	84.7[a]	73.9[b]	80.5[a]	71.0[b]	2.4
Water-soluble Mg	84.9[a]	84.4[a]	64.6[b]	82.9[a]	79.3[a]	55.3[c]	2.5

[1]Nylon bags sewn with nylon thread using sewing machine.

[2]Values are the mean of two observations.

[3]Nylon bags made and sealed by a heat sealer.

[4]Values are the mean of 3 observations.

[abcd]Means on the same row with different superscripts differ ($P<.01$).

Table 28. Losses of particulate DM and water-soluble elements from nylon bags containing 15 different feeds and crop residues, during extraction with water for 8 h, trial 4[1]

	Loss of particulate DM	Water-soluble elements, DM	CP	Ash
Soybean stover	2.9^{j}	12.0^{f}	35.1^{ef}	45.3^{f}
Soybean stalks	3.3^{ij}	8.0^{g}	36.2^{def}	52.8^{d}
Soybean leaves	14.2^{a}	24.0^{abc}	27.9^{h}	28.2^{i}
Soybean pods	2.8^{j}	25.4^{a}	37.3^{de}	76.5^{a}
Corn stover	5.8^{e}	15.4^{e}	38.2^{d}	42.3^{g}
Corn stover silage	5.3^{e}	15.4^{e}	34.5^{f}	44.0^{fg}
Corn husks	3.4^{ij}	3.1^{h}	37.0^{de}	39.1^{f}
Corn stalks	6.8^{d}	10.4^{fg}	44.9^{b}	65.1^{bc}
Corn leaves	5.8^{e}	8.2^{g}	42.5^{c}	25.3^{j}
Oat straw	9.0^{b}	15.4^{e}	56.2^{a}	49.3^{e}
Alfalfa hay	3.7^{ghi}	19.6^{d}	24.2^{i}	64.1^{bc}
Alfalfa, 2nd cut	4.8^{f}	24.9^{ab}	25.9^{hi}	75.1^{a}
Reed canarygrass	3.9^{gh}	23.3^{bc}	34.3^{f}	65.6^{b}
Smooth bromegrass	7.4^{c}	21.8^{cd}	30.9^{a}	45.4^{f}
Tall fescue	4.2^{g}	23.6^{bc}	36.6^{def}	63.5^{c}
Mean	5.6	16.8	36.1	52.1
CV	4.3	6.4	2.9	1.6

[1]Values are the mean of 2 observations.

^{abcdefghij}Means on the same row with different superscripts differ ($P<.05$).

% of total amount initially present				
Ca	P	Mg	K	Mn
46.6^{de}	70.7^{h}	78.0^{c}	99.4^{abc}	48.1^{e}
45.0^{ef}	88.5^{a}	75.8^{d}	98.9^{e}	53.7^{d}
47.1^{d}	55.7^{j}	77.7^{c}	86.4^{h}	12.8^{j}
43.4^{f}	75.5^{f}	82.4^{a}	99.6^{ab}	68.2^{a}
66.9^{a}	78.8^{e}	78.8^{bc}	99.1^{d}	60.5^{b}
68.3^{a}	66.2^{i}	82.6^{a}	98.4^{f}	68.8^{a}
20.2^{i}	75.8^{f}	11.9^{i}	97.5^{g}	11.3^{j}
34.8^{g}	82.1^{d}	43.0^{h}	98.4^{f}	32.4^{h}
30.3^{h}	72.4^{g}	48.6^{g}	98.3^{f}	20.5^{i}
43.6^{f}	87.3^{b}	57.7^{f}	99.3^{cd}	33.1^{h}
35.5^{g}	82.1^{d}	74.9^{d}	99.5^{abc}	45.6^{f}
17.4^{j}	85.0^{c}	69.3^{e}	99.7^{a}	48.6^{e}
60.7^{b}	84.1^{c}	78.1^{bc}	99.6^{ab}	57.7^{c}
53.3^{c}	78.0^{e}	69.4^{e}	99.4^{abc}	38.0^{g}
52.6^{c}	87.3^{b}	79.2^{b}	99.7^{a}	58.3^{c}
44.4	78.0	67.2	98.2	43.8
1.8	.5	.7	.1	2.3

Table 29. Losses of particulate DM and water-soluble elements from nylon bags (dacron cloth) containing 3 different feeds and crop residues, during extraction with water for 8 h, trial 5[1]

	Loss of particulate DM	Water-soluble elements, % of total amount initially present						
		DM	CP	Ash	Ca	P	Mg	Zn
Alfalfa hay	5.9[b]	34.3[a]	40.5[b]	71.6[a]	42.0[b]	90.0[a]	78.6[b]	31.2[a]
Corn stover silage	11.7[a]	13.3[b]	50.8[a]	52.9[b]	54.9[a]	89.6[a]	80.5[a]	16.7[b]
Corn cobs	2.6[c]	6.1[c]	27.0[c]	74.3[a]	13.2[c]	83.0[b]	18.2[c]	14.2[b]
Mean	6.8	17.9	39.4	66.3	36.7	87.5	59.1	20.7
CV	8.0	2.8	8.4	2.6	14.5	1.2	1.8	30.8

[1]Values are the means of 4 observations

[abc]Means on the same column with different superscripts differ ($P<.05$).

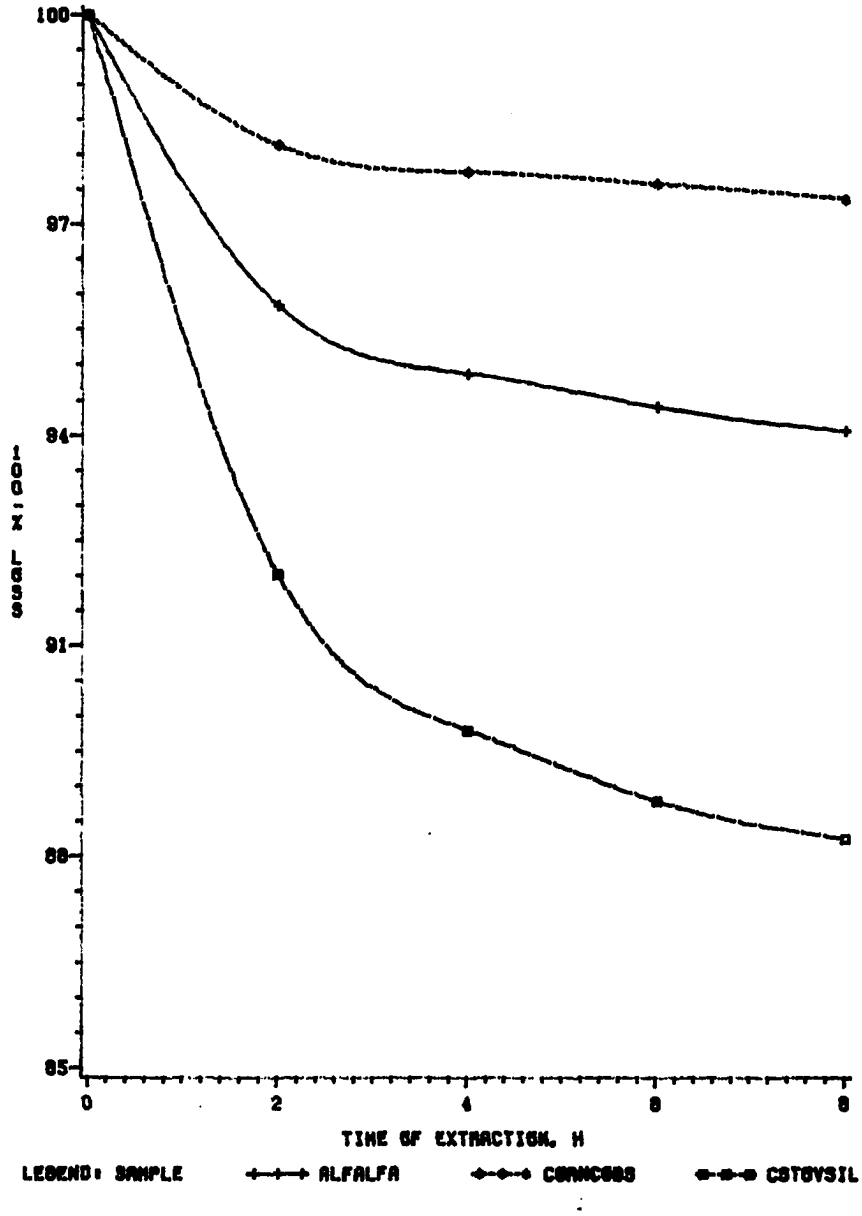

Figure 4. Residual particulate matter (100 - % loss) remaining in dacron bags containing either alfalfa hay, corn cobs or corn stover silage during a 8-h extraction with water

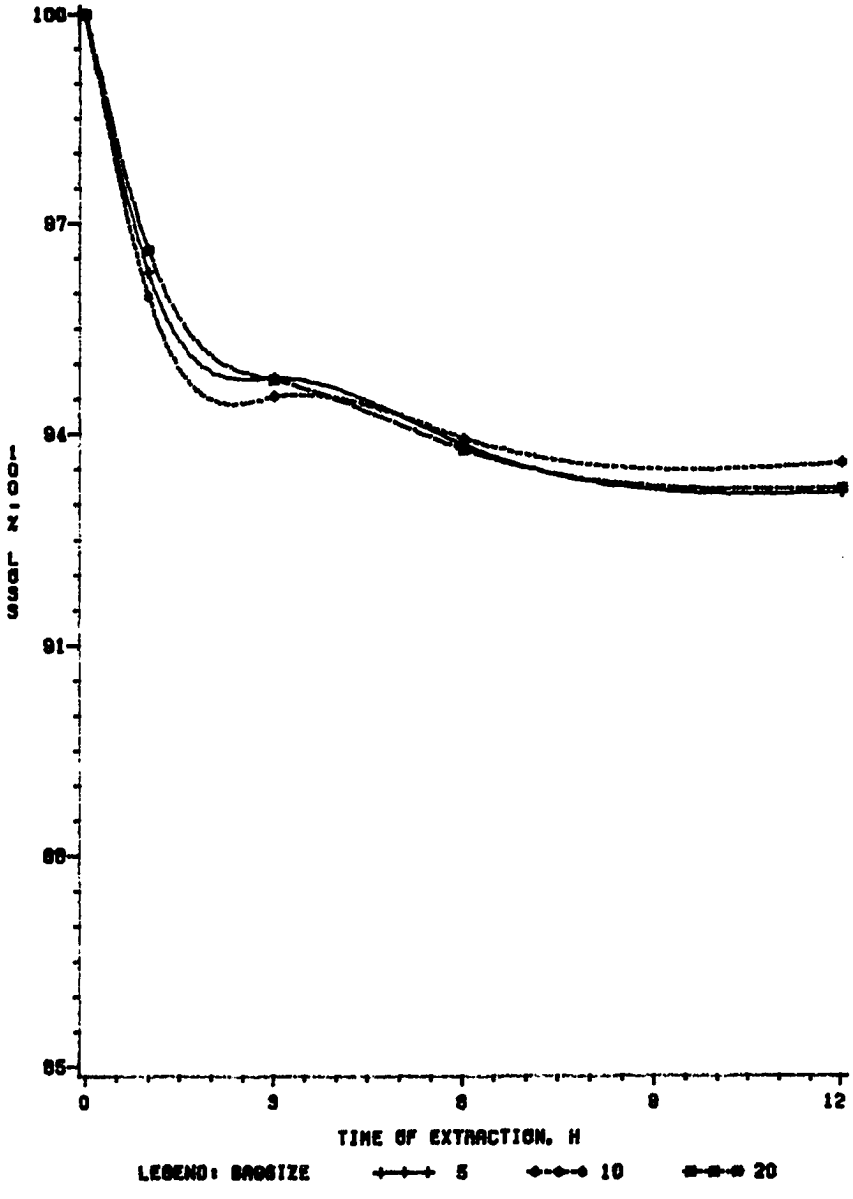

Figure 5. Residual particulate matter (100 - % loss) remaining in dacron bags containing either 5, 10 or 20 mg alfalfa hay/cm^2 of bag surface area during a 12-h extraction with water

particulate matter averaged 4.6% when the samples were milled to pass a 2-mm screen. However, when samples were more finely ground (1 mm), the losses of particulate matter from 25 micron nylon bags increased to about 7% of the original DM.

There was no difference ($P>.80$) between feeds in the loss of particulate matter from bags containing either alfalfa hay or corn stover in trials 1, 2, and 3, when a 20 micron pore size nylon cloth was used. However, when a 30 micron pore size cloth and a larger number of forage feed and crop residues samples (15) were used (trial 4), there were significant differences ($P<.0001$) among feeds and crop residues in trial 5.

Maintaining both the size of the bag (8 x 9 cm) and the amount of the extraction water (150 ml) constant, decreasing the sample size from 3.0 g to either 1.5 g or .75 g caused no change ($P>.41$) in the losses of particulate matter from bags containing alfalfa hay (Figure 5).

Variation in the loss of particulate matter of alfalfa hay through the cloth mesh from day to day was considered small when trials 5 and 6 were compared (Figures 4 and 5). Extrapolating the linear portion of the graph back to the Y-axis, the values of 4.5%, 2.0%, and 8.5% loss of particulate matter would be obtained for alfalfa hay, corn cobs, and corn stover silage, respectively. These values are probably related to the loss of particles smaller than the pore size of

the cloth used. After 3-4 h of shaking, the loss of particulate matter became linear with time. This continued loss of particulate matter for up to 12 h of shaking is not understood.

Breakage of larger particles inside the bag with shaking, therefore making them small enough to go through the cloth mesh, could be an explanation for this continuous increase of the total loss of particulate matter with time.

Playne et al. (1978a) reported on this increase of particulate matter loss with time of extraction when measuring the losses of spear grass from 12 to 72 h in a shaker. They concluded that samples should be extracted for 24 h.

The total loss of particulate matter, shown in Tables 27, 28, and 29, was used as a correction factor for the disappearance of DM in the in situ procedure (nylon bag technique). The technique used to measure the particulate matter loss is an arbitrary one. However, it is repeatable, and appears to simulate what may happen in the rumen, since the losses of water-soluble DM is in agreement with the losses of DM during the first 4-6 h in nylon bags in the rumen.

The total loss of particulate matter, instead of that obtained after 3-4 h, was used because it was understood that probably not only the initial losses would occur in the in situ procedure, but the continuing losses of particulate matter would occur also, after the feeds had been partially digested in the rumen.

The loss of particulate matter was 3.5 times greater in trials 1 and 2 than in trial 3. In the former trials, the bags were sewn with nylon thread using a sewing machine while, in the latter trials, the bags were made and sealed using a heat sealer device. Thus, it seems that a large proportion of the losses observed in trials 1 and 2 occurred through the seam. Therefore, in trials 5 and 6, after the bags were sewn with single stitches using polyester thread, the seams were sealed with Instant Vinyl glue.

The losses of particulate matter through the bag cloth observed in this work is within the range of the values reported in the literature using a similar technique (Playne et al., 1978a; Lindberg and Knutsson, 1981; Lindberg and Varvikko, 1982).

The water-soluble DM, OM, CP, ash, and mineral elements are presented in Tables 27, 28, and 29. The content of CP, ash, and mineral elements in the residue from extraction with water is presented in Appendix Tables A12 and A13, while the analyses of variance of the data are shown in Table B14.

Differences in water-soluble DM among forage feeds and among crop residues were observed ($P<.0001$). The water-soluble DM of forage feeds ranged from 19.6 to 34.3% of the total DM. The average water-soluble DM of the 5 forage feeds shown in Table 28 was 22.2%. Two other alfalfa samples showed 30.2 and 34.3% water-soluble DM, as presented in Tables 27 and 29,

respectively. Todd (1961) reported that the water-soluble DM represented about 25% of the total DM of grasses and clovers, while Playne et al. (1978a) observed that alfalfa hay contained 33.3% water-soluble DM. The values of this fraction of the total DM ranged from 12.6 to 22.4% in tropical grasses and legumes, according to the latter authors.

The water-soluble DM of the crop residues generally was lower than that of the forage feeds. The exceptions to this general trend in the water-soluble DM values were the high levels (25%) of this fraction in soybean leaves and pods as compared to a low level (8%) in soybean stalks. Since recovery of leaves and pods of soybeans in soybean stover is low with current varieties and harvesting methods used, it is doubtful whether these higher water-soluble DM values are important in practical feeding of soybean crop residues to ruminant animals. Vetter (1973) reported that 87% of the total residue in baled stover (Corsoy variety) was soybean stalk.

Water-soluble DM of corn stovers, corn stover silages, and oat straw was about 15% of the total DM. Lindberg and Knutsson (1981) observed that 11.8% of the total DM in straw was water-soluble.

Water extraction, in general, causes a larger disappearance of the ash than of the total DM; in fact, it extracted 3 times more ash than DM, when expressed as a % of its respective initial content (52.1% vs 16.8%). However, only 25-28%

of the ash of both corn and soybean leaves was extracted by this method. Nevertheless, these values are in agreement with the high level of ADF-insoluble ash (75% of the total ash) of corn leaves reported by Vetter (1973).

Differences in water-soluble CP among forage feeds and among crop residues were observed in all trials ($P<.01$). On the average, water extracted twice as much CP as DM, when expressed as a % of initial concentration (Tables 28 and 29). This effect was even more striking with the crop residues and crop residue plant parts than with the forage feeds. The water-soluble CP values of alfalfa and reed canarygrass reported in this work are similar to the 4-h disappearance of N in the rumen from nylon bags, reported by Crawford et al. (1978). These authors reported values of 24.8% and 32.2% for alfalfa hay and reed canarygrass hay, respectively.

The water-soluble P was appreciably higher than the water-soluble Ca of most of the 20 forage feed and crop residue samples studied (Table 27, 28, and 29). This observation is in agreement with the in vivo observations reviewed by Hill (1962) who reported that the availability of P is generally higher than that of Ca of most classes of feedstuffs. The 2 lowest % water-soluble Ca were obtained from corn cobs (13%) and a 2nd cut of alfalfa (17%), while the highest % of water-extractable Ca was observed in corn stover silage (68%). However, a large % of the P present in most samples was ex-

tracted from bags by the water treatment. The values ranged from 66 to 90%, except for soybean leaves, which ranked lowest with 56% water-soluble P. Bromfield and Jones (1972) reported water-soluble P from 60-83% in phalaris and clover plants.

Forage feeds and crop residues containing large amounts of Mg (>.2%) released 2/3 or more of their initial Mg content upon water extraction. However, feeds with smaller amounts of Mg (<.2%) released less than 1/2 of their Mg content during water extraction. Some samples such as corn cobs and corn husks released only 12 to 18% of the initial Mg content under these conditions. This observation is similar to that found by Todd (1961), working with grasses and clovers. The higher release of Mg from feeds containing more than .2% Mg is also in agreement with the review of Kubota et al. (1980), who studied the regional pattern of incidence of grass tetany in the United States. They found that this disease seemed more closely associated with grasses having less than .2% Mg than with grasses having K/Ca + Mg ratios of 2.2 or more. However, the high water-soluble Mg values observed in this work and in that of Todd (1961) are in disagreement with the coefficient of absorption of dietary Mg, presented in Table 5.

Water-soluble K was generally high. It averaged 98.2% of the total K initially present in the bag. The exception was soybean leaves, which had a slightly lower value of 86.4%

water-soluble K.

The microelements (Zn and Mn) generally were extracted with water to a lesser extent than the macroelements.

Size of the bag

The effect of size of the bag (surface area) in relation to weight of the sample was studied in 2 trials. Changing the size of the bag from 6 x 6 cm (40 mg sample/cm^2 of bag surface area) to either a 6 x 9 cm (30 mg/cm^2) or to a 6 x 12 cm (20 mg/cm^2) did not result in any change ($P>.05$) in the disappearance of DM, CP, ash, Ca, P, K, Zn, and Mn of both a 2nd cut of alfalfa and soybean pods fermented for 48 h in the rumen, in trial 1 (Table 30). A small (1.5 digestibility units) but statistically significant increase ($P<.05$) in the disappearance of Mg from bags was observed when the size of the bag was increased from 6 x 6 to 6 x 9 cm. There were significant ($P<.05$) differences between these 2 feeds in relation to the release of all 9 components of DM during digestion in the rumen.

However, in trial 2, when a shorter digestion time (12-48 h) was used, significant differences ($P<.0001$) were observed in DM disappearance among different sizes of bags containing either alfalfa hay or corn stover silage (Figure 6). The ash disappearance of corn stover silage was also affected by the size of the bag (Figure 7). The interactions between type of feed and size of the bag were significant for both DM

Table 30. Effect of size of the bag on the disappearance of DM, CP, ash, Ca, P, Mg, K, Zn, and Mn from 2 feeds suspended in nylon bags[1] in the rumen for 48 h[2]

	Alfalfa, 2nd cut			Soybean pods			Mean		
	20	30	40	20	30	40	ALF	Soy pods	CV
DDM	68.3	68.4	67.7	67.5	66.9	66.4	68.2^{a}	66.9^{b}	1.8
DCP	85.2	85.9	84.4	74.8	77.3	77.3	85.2^{c}	76.5^{d}	1.7
DAsh	88.7	90.0	87.8	89.9	92.9	93.6	88.8^{b}	92.1^{a}	2.8
DCa	74.0	74.8	72.9	85.9	86.3	85.7	73.9^{d}	86.0^{c}	1.2
DP	80.3	82.3	82.3	55.9	58.6	71.2	81.6^{c}	61.9^{d}	9.5
DMg	84.7	86.2	83.5	96.7	96.7	96.1	84.8^{d}	96.5^{c}	.9
DK	99.7	99.7	99.5	99.8	99.9	99.8	99.6^{b}	99.8^{a}	.1
DZn	2.9	18.7	12.2	34.5	29.3	45.9	11.3^{b}	36.6^{a}	76.6
DMn	-31.5	-8.6	-29.5	43.5	55.0	61.2	-23.2^{d}	53.2^{c}	121.4

[1]Either 20, 30, or 40 mg sample DM/cm^{2} of bag surface area.

[2]Values are the means of 4 observations.

[ab]Means in the same row with different superscripts differ ($P<.05$).

[cd]Means in the same row with different superscripts differ ($P<.0001$).

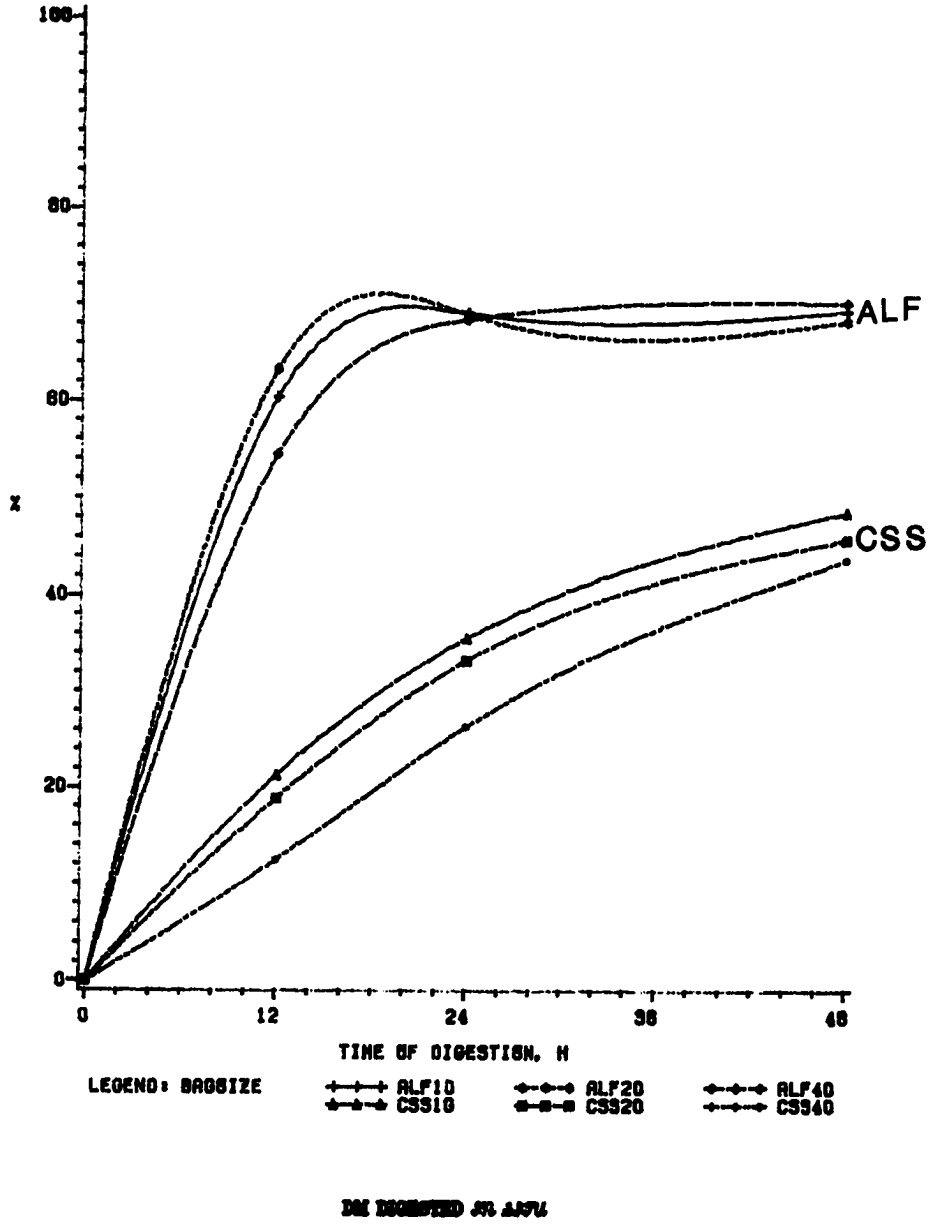

Figure 6. Disappearance of DM of alfalfa hay (ALF) and corn stover silage (CSS) from dacron bags containing 10, 20, and 40 mg sample DM/cm^2 of bag surface area, during digestion in the rumen from 12 to 48 h, expressed as a % of DM initially present

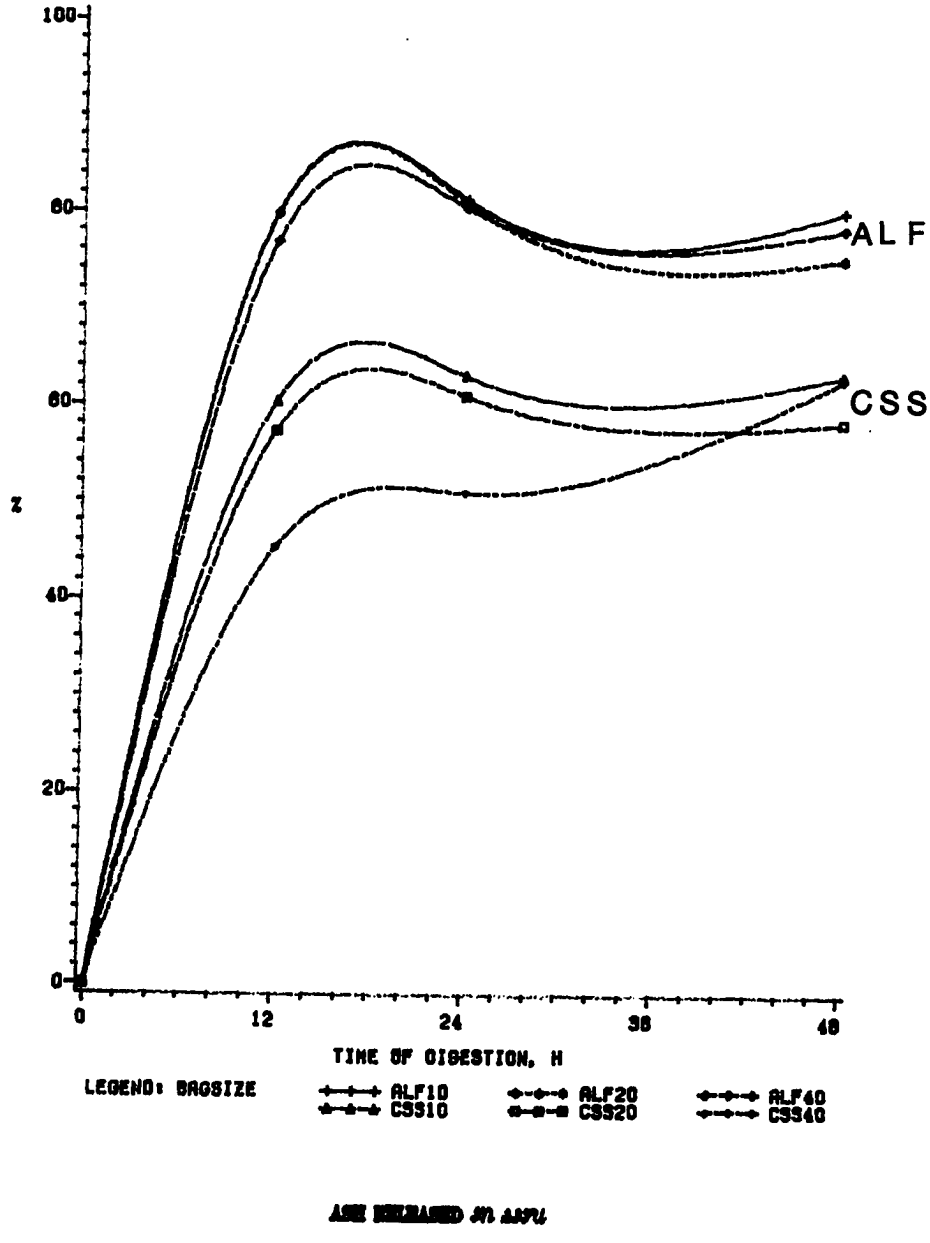

Figure 7. Disappearance of ash of alfalfa hay (ALF) and corn stover silage (CSS) from dacron bags containing 10, 20, and 40 mg sample DM/cm^2 of bag surface area, during digestion in the rumen from 12 to 48 h, expressed as a % of the ash initially present

and ash disappearances from bags, indicating that differences due to size of bag were larger for corn stover silage than for alfalfa hay. In fact, the effect of the size of the bag on the DM digestion of alfalfa hay was significant ($P<.01$) only at the 12-h digestion time while its effect on corn stover silage was significant ($P<.01$) at both 12- and 24-h digestion times.

The lack of effect of the size of the bag when samples were digested for 48 h in situ (Figure 6) is in agreement with the findings of trial 1 (Table 30), where no effect of size of bag was observed on the disappearance of several components of DM. However, when feed samples were incubated in nylon bags in the rumen during shorter periods of time (12-24 h), the disappearance of DM of alfalfa hay and DM and ash of corn stover silage from the smallest size of bag, which corresponds to the largest weight of sample per cm^2 of bag surface area tested (40 mg/cm^2), was lower ($P<.01$) than that from either the 20 or the 10 mg/cm^2 bags.

The results obtained in trial 2 are in agreement with the recommendation of Orskov et al. (1981) that no more than 20 mg sample DM/cm^2 of bag surface area should be used. However, these results are contrary to the findings of Van Hellen and Ellis (1977) and Lindberg (1981) who reported that the best results were obtained with 10 mg sample DM/cm^2 of bag surface area. The disagreement between the 2 different recommendations

is probably related to the pore size of the cloth used. The authors who recommended using a smaller ratio of sample weight to bag surface area (larger size of bag), also used smaller pore size cloth, namely, 10 micron pore size cloth. Nylon cloth having a pore size less than 25 micrometers has been reported to limit the gas release from bags (Uden et al., 1974; Playne et al., 1978a). In this work, gas release was a problem when bags were made of 20 micrometer nylon cloth but not when made of 30 micrometer nylon cloth. Some gas accumulated in dacron polyester bags made of a cloth with a variable pore size (20-75 micrometers).

Type of cloth

The effect of type of cloth on disappearance of DM, CP, ash, Ca, P, Mg, and Zn from either nylon bags or dacron bags containing alfalfa hay suspended in the rumen for either 6, 12, or 24 h is presented in Table 31.

Similar disappearance of DM, CP, P, and Zn of alfalfa hay was obtained with both types of cloth at 6, 12, and 24 h of digestion in the rumen. At 6 h of digestion time, the disappearance of the ash, Ca, and Mg was lower ($P<.01$) from the nylon bags than from the dacron bags. This effect was not observed at either 12 or 24 h digestion times. Thus, it might be that the nylon cloth limited the initial washout of soluble DM constituents, more than the dacron cloth. However, the differences were small and probably not of major importance,

Table 31. Effect of type of cloth on the disappearance of DM, CP, ash, Ca, P, Mg, and Zn from bags containing alfalfa hay digested in the rumen for either 6, 12, or 24 h[1]

	Digestion time, h						
	6		12		24		
	Dacron	Nylon	Dacron	Nylon	Dacron	Nylon	CV
DDM	39.7^{c}	36.8^{c}	60.7^{b}	58.8^{b}	70.1^{a}	66.9^{a}	6.8
DCP	43.6^{c}	41.2^{c}	71.5^{b}	70.0^{b}	82.4^{a}	79.5^{a}	7.9
DAsh	68.7^{b}	65.0^{c}	77.9^{a}	76.9^{a}	79.3^{a}	79.1^{a}	3.1
DCa	41.4^{c}	32.5^{d}	65.1^{b}	63.5^{b}	72.6^{a}	70.2^{ab}	9.5
DP	74.3^{ab}	69.7^{b}	78.2^{ab}	78.0^{ab}	80.7^{a}	81.2^{a}	8.3
DMg	82.3^{c}	79.1^{d}	91.5^{ab}	90.7^{b}	93.7^{a}	93.1^{a}	1.9
DZn	38.0^{c}	35.4^{c}	57.4^{ab}	49.2^{b}	63.3^{a}	56.9^{ab}	14.1

[1]Values are the means of 9 observations.

[abcd]Means in the same row with different superscripts differ ($P<.01$).

biologically speaking.

Therefore, either nylon or dacron cloth, having a pore size between 30 and 50 micrometers, could be used satisfactorily in the *in situ* technique.

Prewashing the bags containing feed samples previous to the rumen digestion

Prewashing the bags containing either alfalfa hay or corn stover silage did not change ($P>.01$) the disappearance of DM, ash, Ca, P, Mg, and Zn during digestion in the rumen (Table 32). Small changes in the disappearance of Mg of alfalfa hay and ash and Ca of corn stover silage were observed in the initial stages (6 h) of digestion.

The contribution of the rumen digestion process to the total disappearance of DM, CP, ash, Ca, P, Mg, and Zn is presented in Table 33. The washout of nutrients from bags was measured by extracting the feed samples with water for 8 h. The residues were exposed in the rumen for 6, 12, and 24 h. The amount of nutrients removed from bags during exposure of the residues of water extraction in the rumen was assumed to be due to digestion. Therefore, the total disappearance of nutrients from bags was assumed to be equal to the sum of the amount released during water extraction and that released during digestion in the rumen.

Rumen digestion contributed to the increase in disappearance of DM, CP, ash, Ca, Mg, and Zn of alfalfa hay, and DM and

Table 32. Effect of prewashing the bags containing either alfalfa hay or corn stover silage on the disappearance of DM, CP, ash, Ca, P, Mg, and Zn from bags containing intact (not washed), and prewashed samples, during digestion in the rumen for 6, 12, or 24 h[1]

	Intact			Prewashed			
	6 h	12 h	24 h	6 h	12 h	24 h	CV
Alfalfa hay							
DDM	39.7^{c}	60.7^{b}	70.1^{a}	38.1^{c}	60.2^{b}	70.3^{a}	8.4
DCP	43.6^{c}	71.5^{b}	82.6^{a}	43.8^{c}	71.3^{b}	81.9^{a}	17.6
DAsh	68.7^{c}	77.9^{a}	79.3^{a}	69.9^{b}	78.7^{a}	80.8^{a}	5.9
DCa	41.4^{c}	65.1^{b}	72.6^{a}	42.1^{c}	66.0^{b}	74.4^{a}	12.3
DP	74.3^{a}	78.2^{a}	80.6^{a}	86.5^{a}	84.7^{a}	85.1^{a}	14.8
DMg	82.3^{c}	91.5^{b}	93.6^{ab}	79.9^{d}	91.8^{b}	94.8^{a}	2.1
DZn	38.0^{be}	57.4^{a}	64.1^{a}	24.3^{c}	53.0^{ab}	59.3^{a}	60.3
Corn stover silage							
DDM	7.2^{c}	18.3^{b}	33.7^{a}	9.2^{c}	20.1^{b}	34.1^{a}	8.4
DCP	20.5^{ab}	10.3^{cd}	8.3^{d}	24.4^{a}	19.8^{abc}	11.2^{bcd}	17.6
DAsh	40.8^{c}	41.7^{c}	45.1^{bc}	46.4^{ab}	49.6^{a}	48.9^{ab}	5.9
DCa	7.2^{e}	23.1^{c}	37.3^{ab}	15.3^{d}	31.0^{b}	38.6^{a}	12.3
DP	50.2^{ab}	33.3^{c}	15.4^{d}	59.2^{a}	41.9^{bc}	19.3^{d}	14.8
DMg	75.3^{d}	78.5^{bc}	81.1^{a}	76.9^{cd}	80.4^{ab}	81.4^{a}	2.1
DZn	9.4^{a}	$-.5^{ab}$	-12.7^{bc}	-1.9^{ab}	-2.4^{ab}	-28.7^{c}	60.3

[1]Values are the means of 9 observations.

[abcde]Means in the same row with different superscripts differ ($P<.01$).

Table 33. Contribution of the rumen digestion to the total disappearance of DM, CP, ash, Ca, P, Mg, and Zn of alfalfa hay and corn stover silage contained in dacron bags suspended in the rumen of fistulated animals following their washing with water[1,2]

	Total disappearance (washout + rumen digestion)			Washout (water-extracted)	Rumen digestion			CV
	6	12	24		6	12	24	
				Alfalfa hay				
DDM	38.1	60.2	70.3	30.9	7.2^{c}	29.3^{b}	39.4^{d}	19.5
DCP	43.8	71.3	81.9	33.4	10.4^{c}	37.9^{b}	48.5^{a}	178.2
DAsh	69.9	78.7	80.8	68.9	1.0^{b}	9.8^{a}	11.9^{a}	92.5
DCa	42.1	66.0	74.4	41.0	1.1^{c}	25.0^{b}	33.4^{a}	302.2
DP	86.5	84.7	85.1	89.2	-2.7^{a}	-4.5^{a}	-4.1^{a}	28.4
DMg	79.9	91.8	94.8	77.0	2.9^{c}	14.8^{b}	17.8^{a}	23.6
DZn	24.3	53.0	59.3	27.6	-3.3^{b}	25.4^{a}	31.7^{a}	246.3
				Corn stover silage				
DDM	9.2	20.1	34.1	8.0	1.2^{c}	12.1^{b}	26.1^{a}	19.5
DCP	24.4	19.8	11.2	41.3	-16.9^{a}	-21.5^{a}	-39.1^{b}	178.2
DAsh	46.4	49.6	48.9	48.2	-1.8^{a}	1.4^{a}	$.7^{a}$	92.5
DCa	15.3	31.0	38.6	43.4	-28.1^{c}	-12.4^{b}	-4.8^{a}	302.2
DP	59.2	41.9	19.3	82.1	-22.9^{b}	-40.2^{a}	-62.8^{c}	28.4
DMg	76.9	80.4	81.4	76.9	0.0^{b}	3.5^{a}	4.5^{a}	23.6
DZn	-1.9	-2.4	-28.7	19.3	-21.2^{a}	-21.7^{a}	-48.0^{b}	246.3

[1]Values are the means of 9 observations.

[2]Trial conducted at ISU with fistulated animals fed a mixture of whole plant corn silage and corn stover silage.

[abc]Means on the same row with different superscripts differ ($P<.01$).

Mg of corn stover silage. However, a larger amount (weight) of CP, Ca, P, and Zn was found in the residue of digestion of corn stover silage in the rumen than that which was initially present in the bag. Therefore, the rumen digestion process seemed to have contaminated the residues of digestion of feed samples. Mathers and Aitchison (1981) found that microbial contamination of feed residues increased linearly with time in the rumen. They reported that about 1/5 of the residual N in lucerne samples was of microbial origin after 48 h in the rumen.

When the contribution of the rumen digestion was calculated for the 15 feeds and crop residues digested for 48 h in the rumen of Holstein-cross-bred steers (in Brazil), somewhat different results were obtained (Table 34). The diet of the fistulated animals was similar in both locations.

Like in the ISU study, some crop residues were contaminated with CP and P. No Ca contamination was observed in the Brazilian study in contrast to that observed in the ISU study. The largest difference between the two studies seemed to be related to the extent of contamination of the residues with microbial matter. This contamination was even more striking when it was calculated for corn cobs. After 24 h of rumen digestion, the contamination was 166% and 343% of the initial levels of CP and P, respectively. The initial composition of the corn cobs was similar to that of the corn husks used in

Table 34. Calculated contribution of the rumen digestion[a] to the total disappearance of DM, CP, ash, Ca, P, Mg, and Mn of 15 feeds and crop residues suspended in nylon bags in the rumen for 48 h[b]

Feeds and crop residues	DM	CP	Ash	Ca	P	Mg	Mn
	% of amount initially present						
Soybean stover	24.8	30.3	22.6	32.1	-2.3	15.2	4.2
Soybean stalks	21.4	5.1	18.4	36.9	-15.0	16.8	-22.5
Soybean leaves	34.5	33.1	25.1	38.7	9.7	11.6	39.7
Soybean pods	33.7	39.4	14.8	38.8	15.6	15.4	5.3
Corn stover	29.8	12.4	13.1	12.9	-13.0	14.4	15.9
Corn stover silage	29.3	14.2	17.3	15.9	.6	10.9	1.1
Corn husks	36.4	-16.0	9.8	22.8	-51.5	66.1	60.1
Cornstalks	24.7	-14.0	2.0	12.1	-21.0	33.4	31.5
Corn leaves	34.3	4.7	15.8	45.2	-6.9	39.9	62.9
Oat straw	24.2	-6.3	5.3	26.2	-5.4	29.4	19.5
Alfalfa hay	39.5	59.0	16.0	47.7	7.3	18.3	3.9
Alfalfa, 2nd cut	42.3	60.3	14.5	54.7	7.4	21.8	-20.4
Reed canarygrass	39.6	50.3	5.7	11.3	8.2	15.3	26.9
Smooth bromegrass	36.0	35.8	8.8	26.4	10.1	17.8	30.1
Tall fescue	39.8	45.0	6.4	24.0	4.2	14.1	23.1

[a]Rumen digestion = total disappearance - washout.

[b]Values are the means of 8 observations. Trial conducted in Brazil with fistulated animal fed whole plant corn silage-based diet.

the Brazilian study. Corn husks showed a considerably smaller contamination than corn cobs, i.e., 16% and 52% of the initial levels of CP and P, respectively.

While the washing of the residues might have been partially responsible for the difference in contamination of the residues between the 2 studies, a different microbial population in the rumen of the ISU animals compared with the Brazilian animals also could account for some of the difference in the contamination observed. Conceivably, it is possible to have a larger proportion of rumen microorganisms which adhere to cell walls of feedstuffs during digestion in the American animals than in the Brazilian animals. Therefore, it would be more difficult to wash the microorganisms away from the bags containing the undigested feed residues fermented in the rumen of the former animals than in the latter ones.

Final adopted nylon bag technique

The disappearance of the total DM and 6 other components (CP, ash, Ca, P, Mg, Mn) of the DM from feeds and crop residues digested in the rumen in trials 1 and 2 is shown in Figures 8 through 18. The content of CP, ash, Ca, P, Mg, and Mn in the residue of digestion in the rumen is presented in Appendix Tables A14 and A15. The linear and quadratic regression analysis of the data is presented in Appendix Table B15.

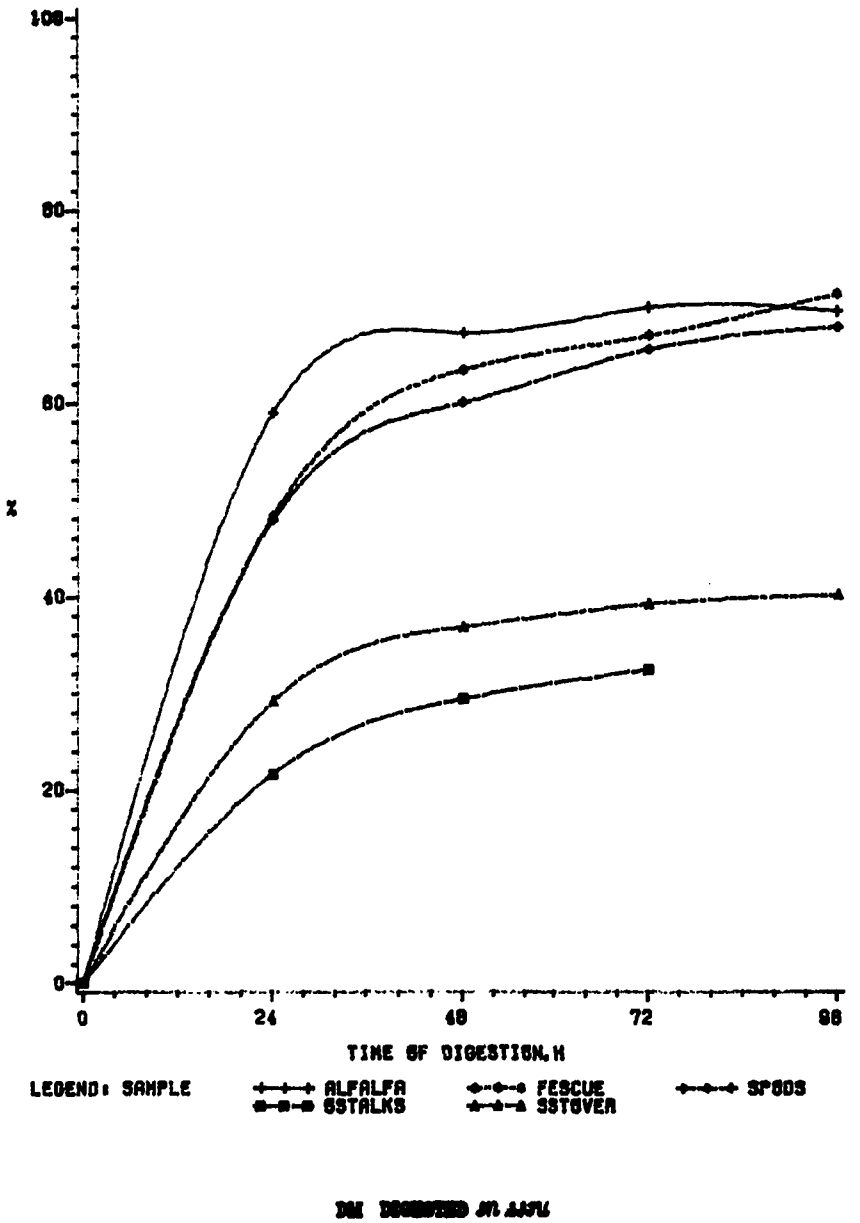

Figure 8. DM disappearance (DM digested in situ) of alfalfa (alfalfa, 2nd cut), fescue (tall fescue), spods (soybean pods), sstalks (soybean stalks), and sstover (soybean stover) in nylon bags in the rumen, expressed as % of DM initially present

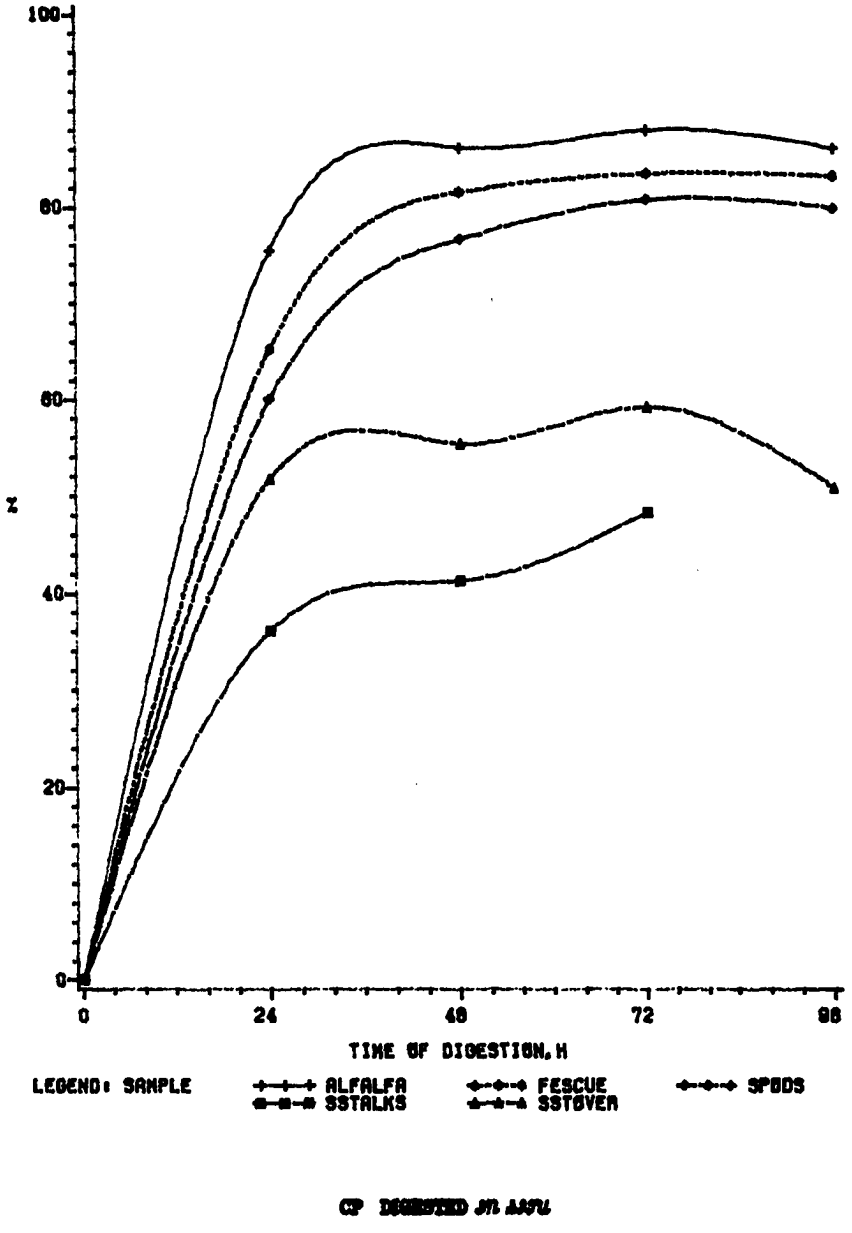

Figure 9. CP disappearance (CP digested *in situ*) of alfalfa (alfalfa, 2nd cut), fescue (tall fescue), spods (soybean pods), sstalks (soybean stalks), and sstover (soybean stover) in nylon bags in the rumen, expressed as % of CP initially present

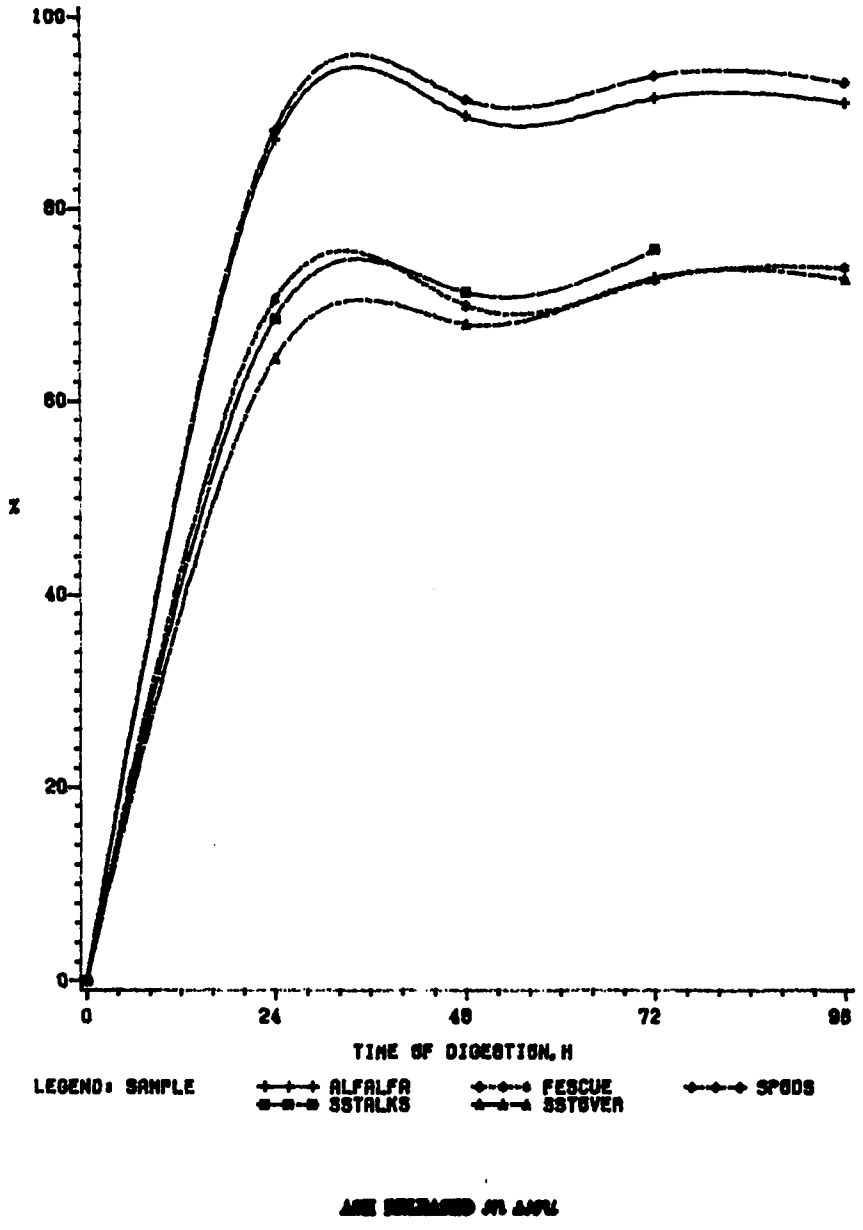

Figure 10. Ash disappearance (ash release in situ) of alfalfa (alfalfa, 2nd cut), fescue (tall fescue), spods (soybean pods), sstalks (soybean stalks), and sstover (soybean stover) in nylon bags in the rumen, expressed as % of ash initially present

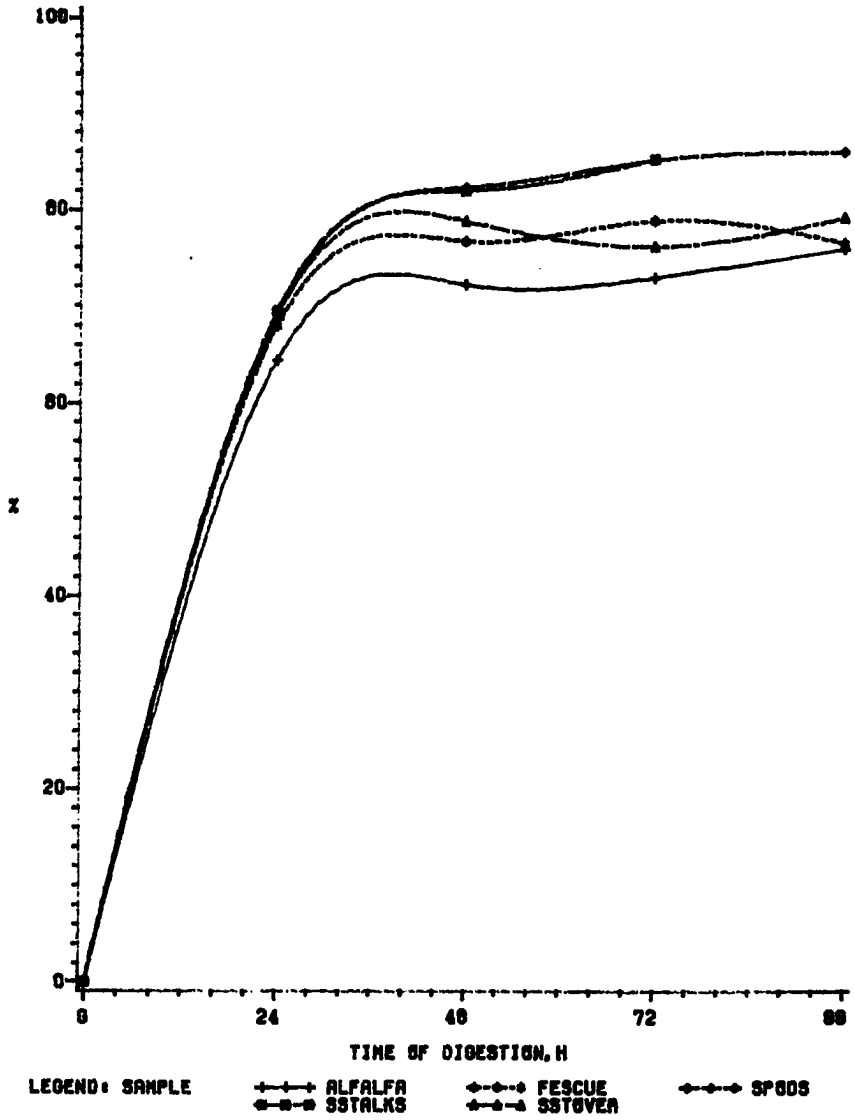

Figure 11. Ca disappearance (Ca released *in situ*) of alfalfa (alfalfa, 2nd cut), fescue (tall fescue), spods (soybean pods), sstalks (soybean stalks), and sstover (soybean stover) in nylon bags in the rumen, expressed as % of Ca initially present

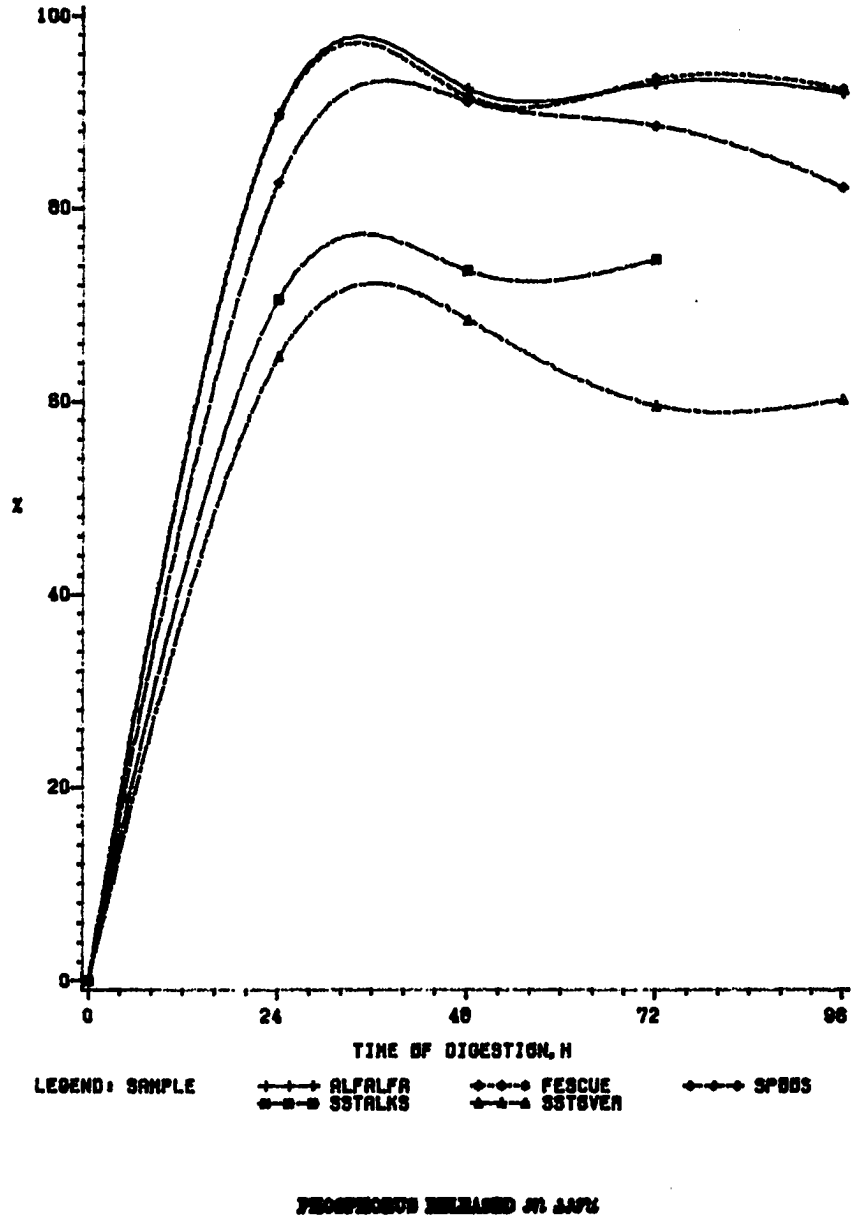

Figure 12. P disappearance (P released *in situ*) of alfalfa (alfalfa, 2nd cut), fescue (tall fescue), spods soybean pods), sstalks (soybean stalks), and sstover (soybean stover) in nylon bags in the rumen, expressed as % of P initially present

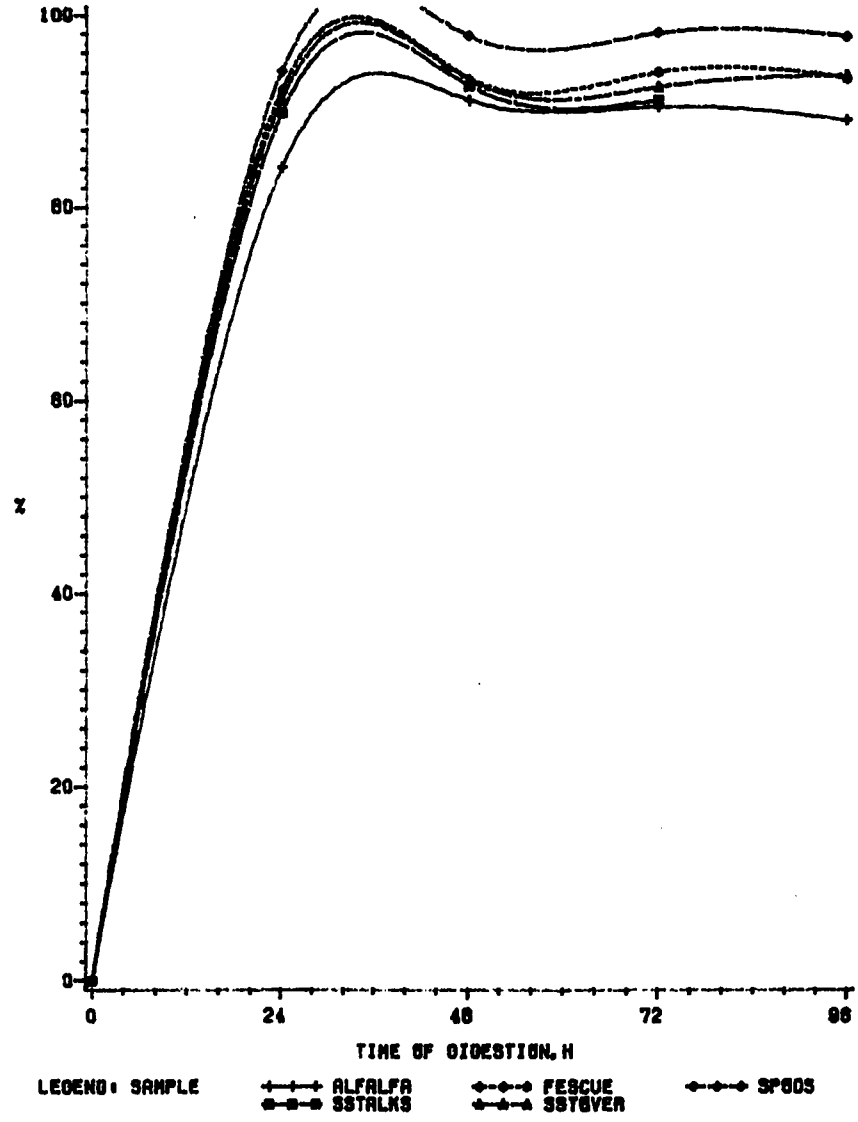

Figure 13. Mg disappearance (Mg released *in situ*) of alfalfa (alfalfa, 2nd cut), fescue (tall fescue), spods (soybean pods), sstalks (soybean stalks), and sstover (soybean stover) in nylon bags in the rumen, expressed as % of Mg initially present

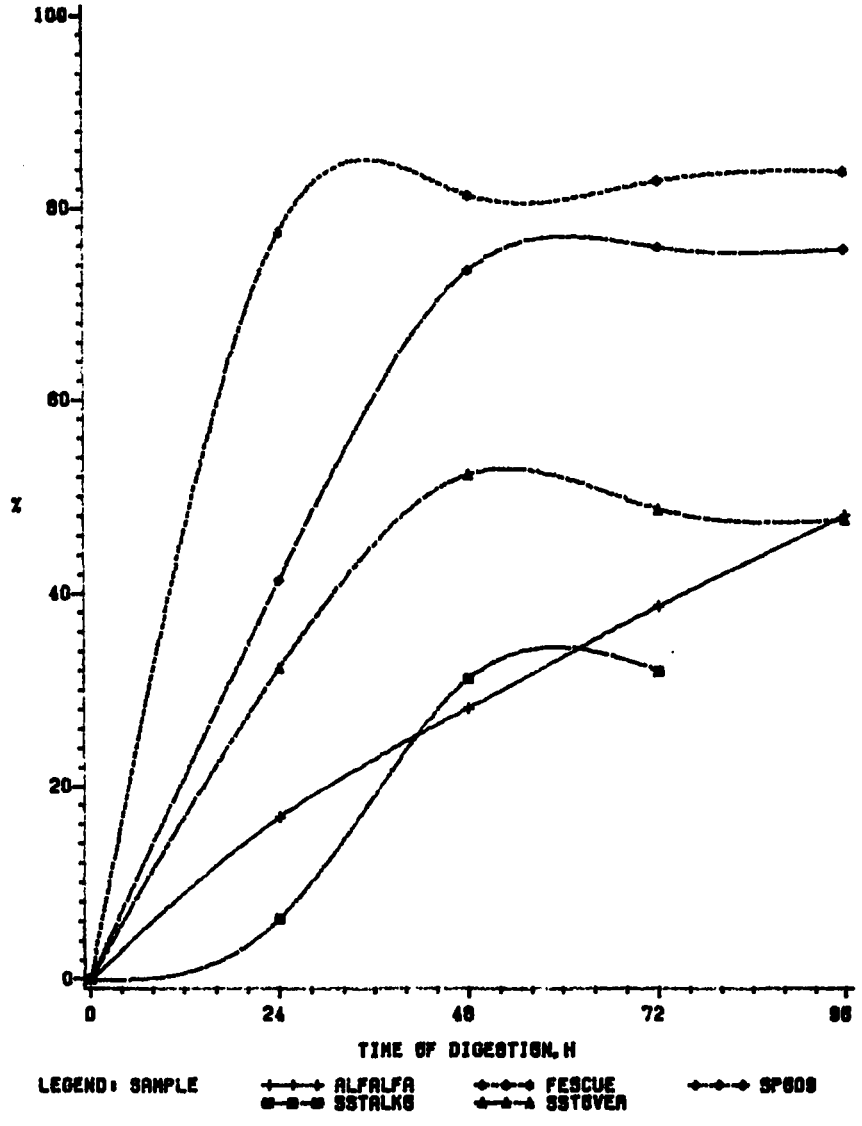

Figure 14. Mn disappearance (Mn released in situ) of alfalfa (alfalfa, 2nd cut), fescue (tall fescue), spods (soybean pods), sstalks (soybean stalks), and sstover (soybean stover) in nylon bags in the rumen, expressed as % of Mn initially present

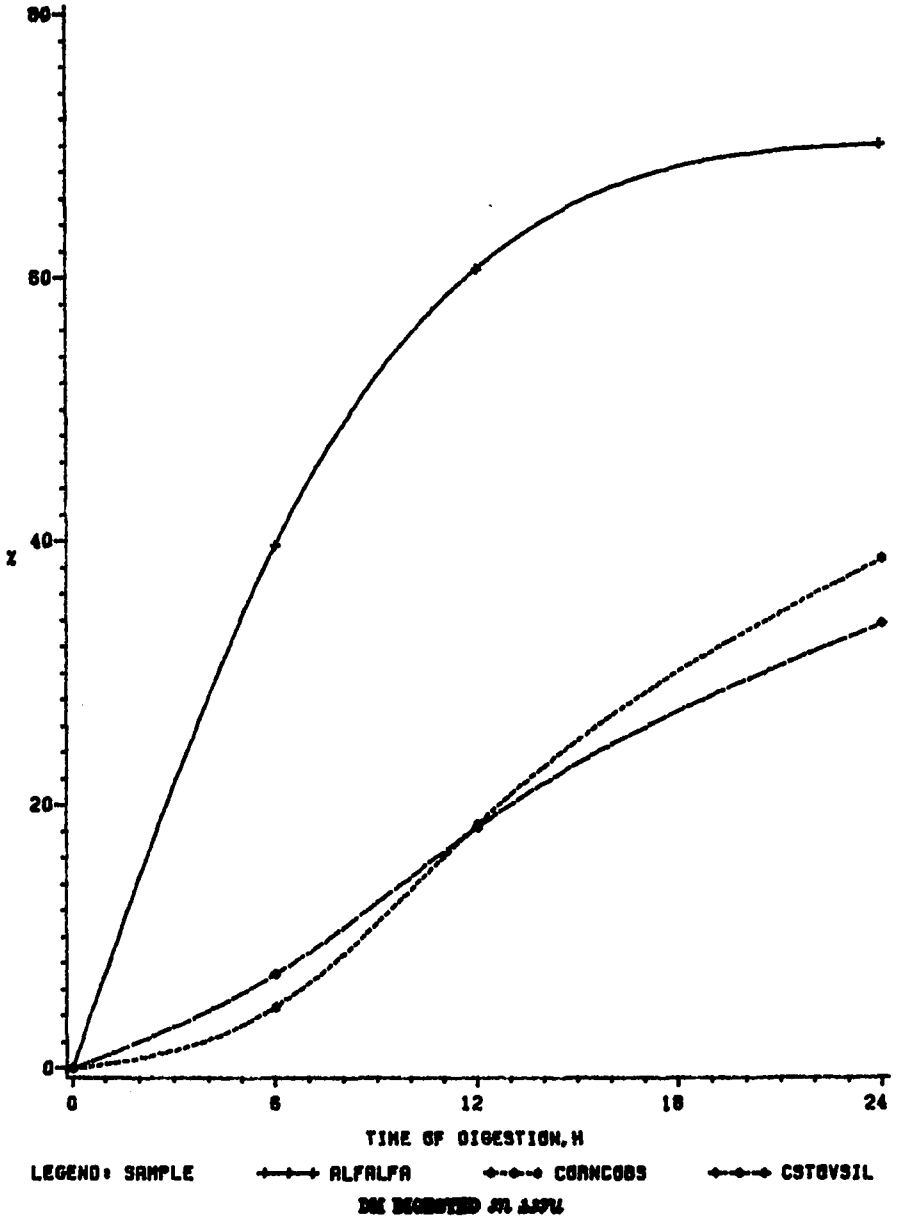

Figure 15. DM disappearance (DM digested in situ) of alfalfa (alfalfa hay), corncobs (corn cobs) and cstovsil (corn stover silage) in dacron bags in the rumen, expressed as % of DM initially present

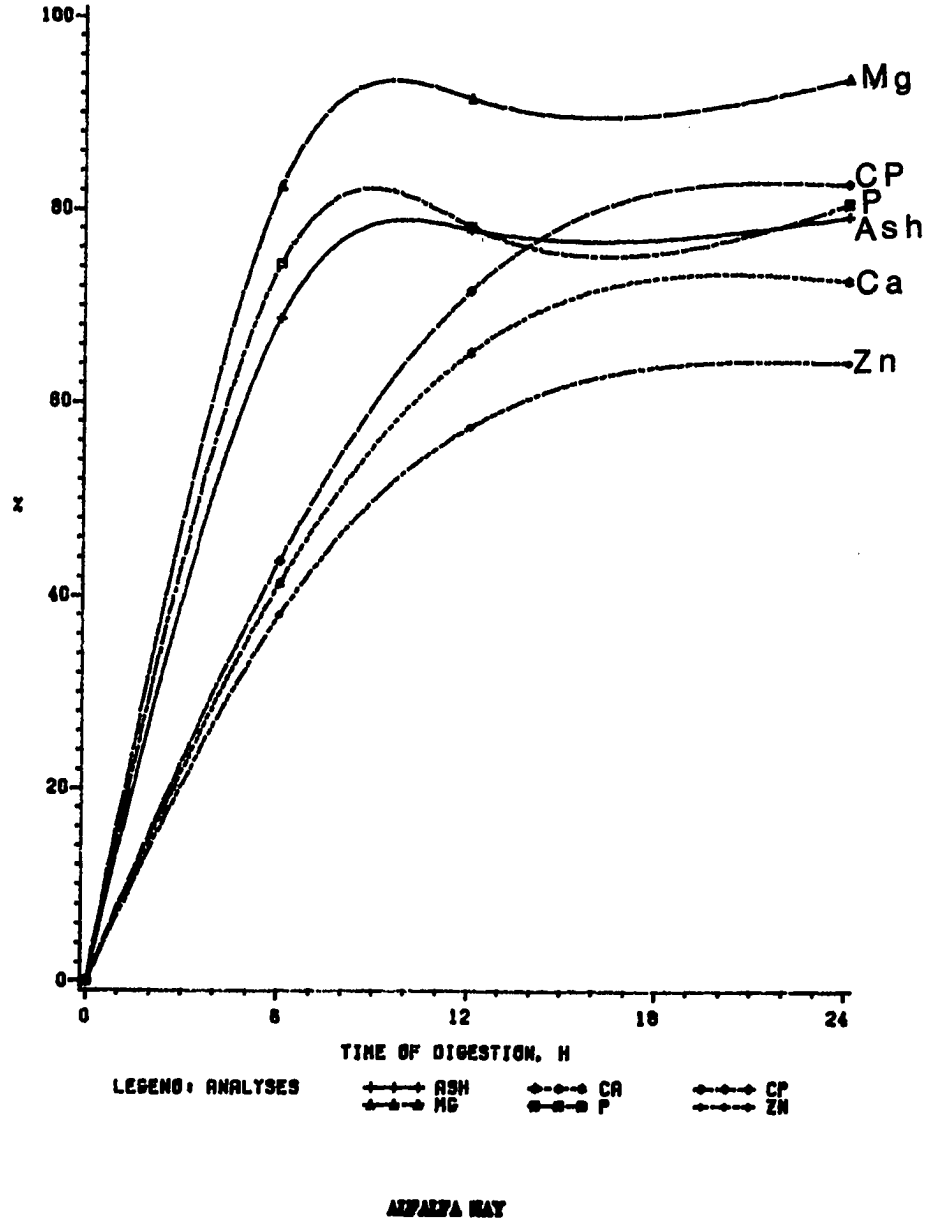

Figure 16. Ash, CA (Ca), CP, MG (Mg), P, and ZN (Zn) disappearance from dacron bags containing alfalfa hay during digestion in the rumen, expressed as % of the respective component initially present

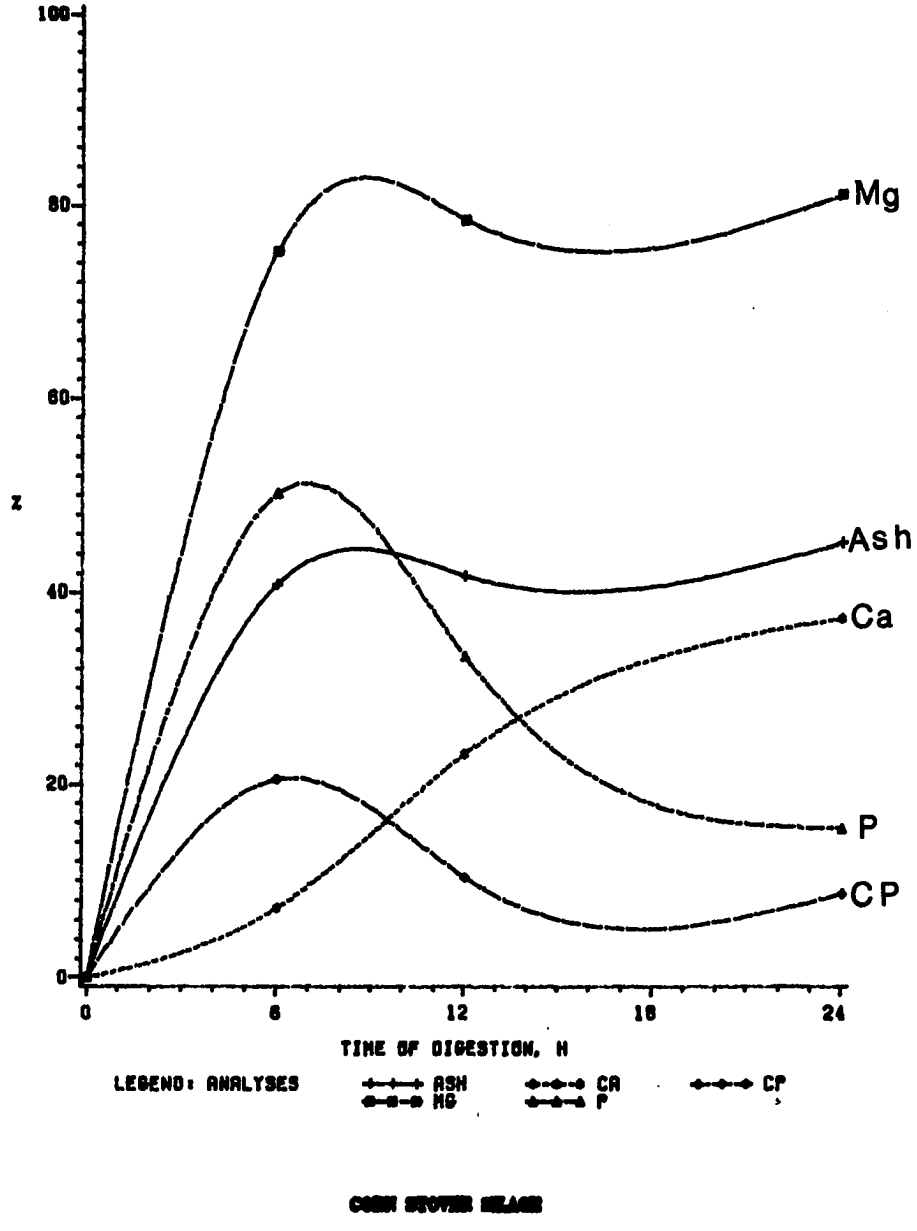

Figure 17. Ash, CA (Ca), CP, MG (Mg), and P disappearance from dacron bags containing corn stover silage during digestion in the rumen, expressed as a % of the respective component initially present

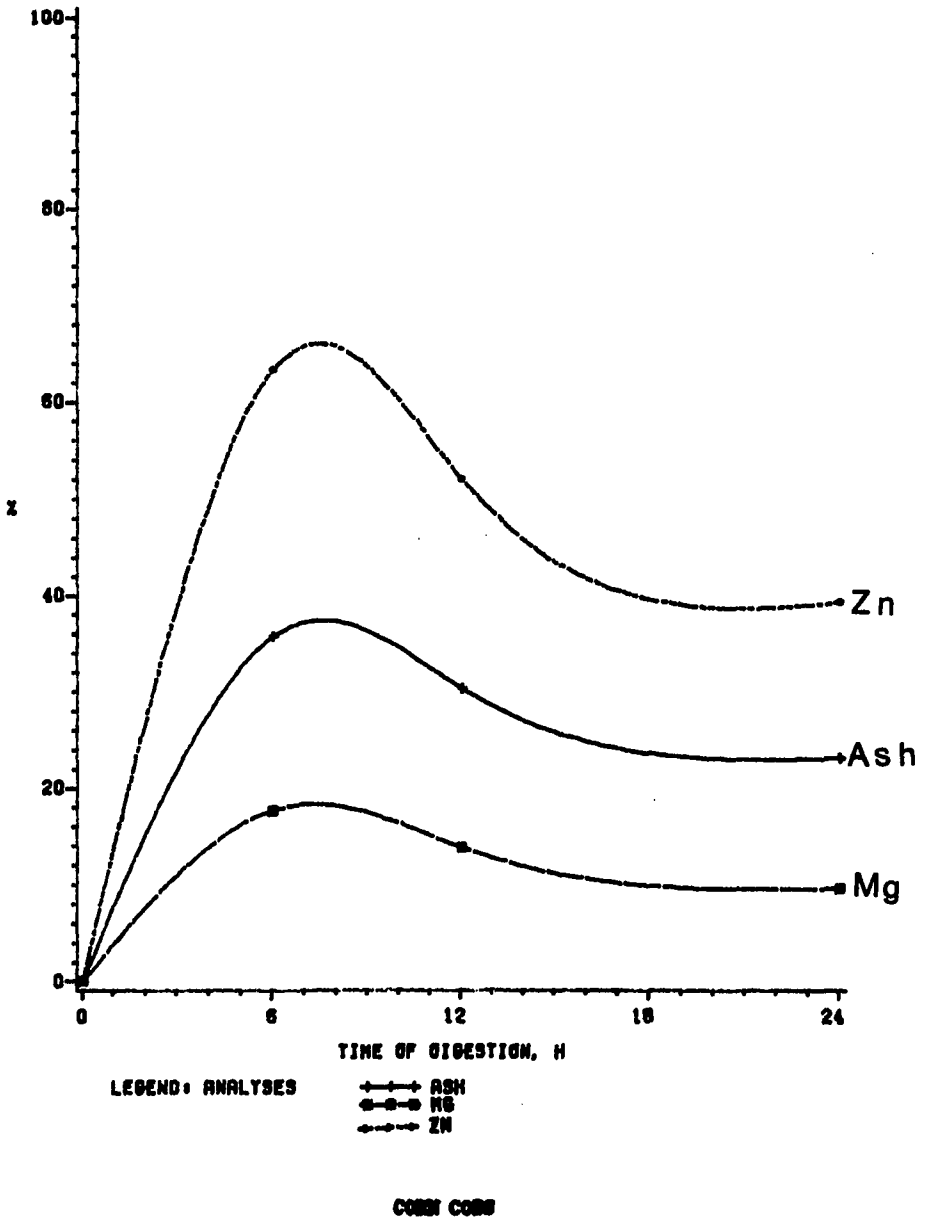

Figure 18. Ash, MG (Mg), and ZN (Zn) disappearance from dacron bags containing corn cobs during digestion in the rumen, expressed as a % of the respective component initially present

Results presented in Figures 8 through 14 referred to 5 of the 8 forage feeds and crop residues tested from 24 to 96 h in nylon bags in the rumen. The results of this trial are also presented in Appendix Table A16.

It can be seen from Figures 8 through 14 and Appendix Table A16 that disappearance of all 7 components (DM, CP, ash, Ca, P, Mg, Mn) studied increased ($P<.01$) with time of digestion in nylon bags in the rumen. The time of maximum disappearance of each element differed. For example, DM and Mn disappearance continued to increase up to the longest time (96 h) studied while that of CP, ash, Ca, and P increased up to 72 h and Mg increased up to 48 h in the rumen. However, increases of disappearance of all elements studied after 48 h of digestion time were small, and probably have no biological importance. In fact, the largest portion of the disappearance of most elements occurred in the first 24 h of exposure in the rumen. Therefore, disappearance of DM, CP, ash, Ca, P, Mg and Zn from dacron bags containing either alfalfa hay, corn stover silage or corn cobs were measured during either 6, 12, or 24 h in the rumen in trial 2 (Figures 15 through 18 and Appendix Table A17).

Like in trial 1, DM disappearance from bags containing all 3 feeds increased ($P<.01$) with time of digestion in the rumen. The rate of DM disappearance of alfalfa hay in trial 2 was higher than that of other alfalfa samples in trial 1.

Even though part of this difference in DM disappearance could be due to sample, another factor which might be partially responsible for the difference in the release of DM from bags was the size of bags, as explained in the previous section.

The CP, Ca and Zn disappearance from bags containing alfalfa hay increased ($P<.01$) with length of time of exposure for digestion in the rumen. The shape of the curve was similar to that of the DM disappearance of alfalfa hay. The ash and P disappearance from this feed sample was not significantly affected ($P>.01$) by length of time of digestion. A small but significant effect ($P<.01$) of time of digestion was observed for the Mg disappearance of alfalfa hay and corn stover silage.

The Ca disappearance from the corn stover silage was similar to that of the DM. However, an opposite response was observed for CP and P. It is possible that this was due to contamination of the residues with microbial matter (Mathers and Aitchison, 1981). An increased contamination with longer digestion time in the rumen would explain the lower ($P<.01$) disappearance of CP and P from bags containing corn stover silage. Also, it would explain the lower ($P<.01$) disappearance of CP, ash, P, Mg, and Zn in corn cobs with longer digestion time in the rumen.

Some difference in the extent of contamination between trial 1 (Brazil) and trial 2 (ISU) was observed. While in

trial 1, it was possible to measure the contamination only after 96 h of digestion (Appendix Table A16), in trial 2, the contamination was measurable after 6 to 12 h of digestion in the rumen (Appendix Table A17).

A comparison of 14 feeds and crop residues, using the *in situ* procedure is presented in Table 35. Exposing the samples in nylon bags in the rumen during 48 h resulted in higher ($P<.0001$) disappearance of DM, CP, ash, Ca, P, Mg, and Mn than when exposing them during 24 h. The effect of digestion time on the disappearance of the ash, P, and Mg was small.

The 48-h disappearance of DM observed for corn stover (45.2%), corn stover silage (44.7%), oat straw (39.6%), and soybean stover (36.8%) was within the range of DMD (DM digestibility) of these crop residues observed in the *in vivo* digestibility study with sheep (Elhag, 1976). The values of DMD (ranges given in parentheses) of corn stover, corn stover silage, oat straw, and soybean stover were 35.6 (25.9-45.2), 46.7 (42.5-51.0), 43.1 (36.5-54.2), and 38.2 (30.2-48.2), respectively.

The disappearance of ash was generally higher than that of the DM which would indicate that the disappearance of OM *in situ* was lower than that of DM. After 48 h exposure in the rumen, 73% of the ash initially present in the 5 forage feeds had disappeared from the bags. This value is in agreement with the 71% *in vitro* disappearance of the ash of 6

Table 35. Disappearance of DM, CP, ash, Ca, P, Mg, and Mn of 14 feeds and crop residues contained in nylon bags suspended in the rumen of fistulated animals for either 24 or 48 h[a]

Sample	DM		CP		Ash	
	24	48	24	48	24	48
	Time of digestion, h					
Soybean stover	29.1	36.8	51.7	55.4	64.4	67.9
Soybean stalks	21.6	29.4	36.1	41.3	68.5	71.2
Soybean leaves	50.0	58.5	51.2	61.0	50.1	53.3
Soybean pods	47.8	60.1	60.0	76.7	88.1	91.3
Corn stover	32.9	45.2	36.4	50.6	52.3	55.4
Corn stover silage	30.8	44.7	34.9	48.7	56.4	61.3
Corn husks	23.6	39.5	15.8	21.1	45.7	48.9
Corn leaves	29.7	42.5	34.2	47.2	36.0	41.1
Oat straw	26.1	39.6	46.8	49.9	53.5	54.6
Alfalfa hay	49.5	59.1	69.7	83.2	78.5	80.1
Alfalfa, 2nd cut	58.9	67.2	75.5	86.2	87.1	89.6
Reed canarygrass	51.3	62.9	71.6	84.6	68.2	71.3
Smooth bromegrass	41.6	57.8	41.0	66.7	49.7	54.2
Tall fescue	48.2	63.4	65.2	81.6	70.5	69.8
Mean	38.6	50.5	49.3	61.0	62.1	65.0

[a]Values are the means of 8 observations.

Ca		P		Mg		Mn	
24	48	24	48	24	48	24	48
Time of digestion, h							
68.9	78.7	64.6	68.4	91.1	93.2	32.3	52.3
69.4	81.9	70.6	73.5	89.7	92.6	6.3	31.2
78.8	85.8	62.5	65.4	86.2	89.2	49.6	52.5
69.1	82.2	82.6	91.1	94.1	97.7	41.4	73.5
78.1	79.8	66.4	65.8	91.6	93.2	71.2	76.4
75.3	84.2	65.4	66.8	89.8	93.5	60.0	69.9
10.0	43.0	23.5	24.3	65.2	78.0	63.0	71.4
63.5	75.5	64.8	65.5	85.9	88.5	75.5	83.4
54.8	69.8	85.2	81.9	78.9	87.1	26.5	52.6
75.6	83.2	86.9	89.4	85.8	93.2	32.2	49.5
64.4	72.1	89.8	92.4	84.1	91.1	16.8	28.2
63.2	72.0	89.9	92.3	89.6	93.4	77.9	84.6
62.8	79.7	81.7	88.1	82.2	87.2	51.4	68.1
67.9	76.6	89.6	91.5	92.2	93.3	77.5	81.4
64.4	76.0	73.1	75.5	86.2	90.8	48.7	62.5

tropical hays (McLeod and Minson, 1974). The higher disappearance of DM than OM is in contrast to most *in vivo* digestibility studies where the digestibility of OM is generally higher than that of DM (McLeod and Minson, 1974). The *in vivo* OM digestibility was higher than DM digestibility of both whole plant corn silage and elephant grass silage (Table 37).

Digestion of crop residues *in situ* contributed to the release of most mineral elements studied; that is, a larger proportion of the elements were removed during digestion in nylon bags in the rumen than in a simple water extraction process. Phosphorus was the exception to this general rule. The effect on the CP disappearance was small. Therefore, the largest portion of the CP and P in crop residues which is potentially available to the rumen microorganisms and to the host animal seemed to be associated with the water-soluble fraction. On the other hand, digestion of forage feeds seemed to have contributed to removing all mineral elements studied. The largest effect of digestion of DM of forage feeds seemed to be on the removal of CP, Ca and microelements (Mn and Zn). The lowest effect was observed on P and Mg removal from bags. Water extraction removed 92% and 81% of the P and Mg removed (available) during a 48-h digestion *in situ*.

The 86% disappearance of CP in the 2nd cut of alfalfa was similar to 78% disappearance of CP in alfalfa hay reported by

Playne et al. (1978b), using a similar *in situ* technique. They also reported 65% release of Ca from alfalfa hay while, in this study, 72% of the Ca in a 2nd cut of alfalfa was removed during digestion in the rumen. Armstrong and Thomas (1952) reported a higher (84.9%) availability of Ca in alfalfa to rats.

The proportion of the total Ca not removed from bags containing alfalfa samples during digestion in the rumen is in agreement with the average level of oxalate in this plant reported by Ward et al. (1979). These authors reported that oxalate was equivalent to 24% of the total calcium present in this forage feed.

The high potential availability of Ca in alfalfa is in contrast to the low true absorption (31 to 41%) of this element observed in steers (Hansard et al., 1957). However, this low true absorption of Ca *in vivo* seemed to be due to the high level of Ca intake (twice the maintenance requirement) while the DM intake was set at about maintenance level. Moreover, absorption of Ca seems to be regulated at the intestinal level. Thus, the animal can control the efficiency of Ca absorption to meet a change in requirement (Scott and McLean, 1981; Abdel-Hafeez et al., 1982; Braithwaite, 1978a).

The 92% disappearance of P of 2nd cut of alfalfa is similar to the *in vivo* true absorption of P obtained by Lofgreen and Kleiber (1954).

The average release of Mn was the lowest among the 4 mineral elements (Ca, P, Mg, Mn) studied (Table 35). The forage grasses released a greater % of the initial content of Mn than did the forage legumes (78% vs 39%). This was also true when the corn crop residues were compared with the soybean crop residues (71% vs 52%).

Apparent and Calculated *in vivo* True Digestibility of Silages Fed to Sheep with 4 Levels of Added Mineral Elements

The intake of DM, CP, and mineral elements from whole plant corn silage and elephant grass silage and intake from the concentrate-mineral mixtures fed to sheep in metabolism cages are presented in Table 36. The apparent digestibility of DM, OM, gross energy, and CP, and the apparent absorption of ash, Ca, P, Mg, Zn, Mn, and Cu, and nitrogen balance of the sheep fed the 8 combinations of silages and mineral mixtures are shown in Table 37. The calculated true absorption of Ca, P, Mg, Zn, and Cu of either whole plant corn silage or elephant grass silage supplemented with each level of mineral elements is presented in Table 38. The content of CP, ash, Ca, P, Mg, Zn, Mn, and Cu in the feces of sheep fed each one of the 8 diets is presented in Appendix Table A18. The analysis of variance tables are shown in Appendix Tables B16, B17, and B18.

The intake of DM, and mineral elements (Ca, P, Mg, Zn,

Table 36. Intake of DM, CP, and mineral elements from silages and concentrate-mineral mixtures fed to sheep in metabolism cages[1,2]

	Corn silage				Elephant grass silage				
	0	1	2	3	0	1	2[3]	3	CV
DM intake									
Silage, Kg/day	.86[a]	.91[a]	.95[a]	.90[a]	.78[b]	.69[b]	.71[b]	.75[b]	14.4
Min. mix., Kg/day	.18	.18	.18	.18	.18	.18	.18	.18	
Total, Kg/day	1.03[a]	1.09	1.12[a]	1.07[a]	.96[b]	.86[b]	.88[b]	.93[b]	11.8
Total, g/Kg$^{.75}$	56.8[a]	59.7[a]	62.5[a]	63.0[a]	55.0[b]	52.0[b]	51.4[b]	53.1[b]	11.3
Total CP intake, g/day	95.0	98.3	97.0	98.7	70.0	66.0	67.5	70.3	
Mineral requirement, intake and excretion									
Ca, g/day									
Requirement	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	
Intake - silage	1.10[b]	1.17[b]	1.21[b]	1.15[b]	2.80[a]	2.50[a]	2.30[a]	2.66[a]	16.0
Intake - min. mix.	.05	1.25	2.67	6.14	.05	1.25	2.67	6.14	
Total	1.15[f]	2.42[e]	3.88[d]	7.29[b]	2.85[e]	3.75[d]	4.97[c]	5.80[a]	6.8
Fecal excretion	1.17[g]	2.04[g]	3.08[de]	6.22[b]	2.76[e]	3.59[d]	4.32[c]	8.25[a]	9.4
P, g/day									
Requirement	2.80	2.80	2.80	2.80	2.80	2.80	2.80	2.80	
Intake - silage	1.30[ab]	1.39[ab]	1.44	1.36[ab]	1.29[ab]	1.14[ab]	1.03[b]	1.23[ab]	16.5
Intake - min. mix.	.51	1.37	2.42	3.49	.51	1.37	2.42	3.49	
Total	1.81[e]	2.76[d]	3.86[b]	4.85[a]	1.80[e]	2.51[d]	3.45[c]	4.72[a]	6.5
Fecal excretion	1.55[e]	2.34[d]	3.23[b]	4.06[a]	1.75[de]	2.40[cd]	3.02[bc]	4.60[a]	13.2

Mg, g/day									
Requirement	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52	
Intake - silage	1.38[a]	1.47[a]	1.53[a]	1.44[a]	1.83[a]	1.62[a]	1.47[a]	1.74[a]	16.3
Intake - min. mix.	.18	.23	.28	.32	.18	.23	.28	.32	
Total	1.56[a]	1.70[a]	1.81[a]	1.76[a]	2.01[a]	1.85[a]	1.75[a]	2.06[a]	14.1
Fecal excretion	.65[e]	.79[de]	1.00[cd]	.99[cd]	1.45[ab]	1.36[ab]	1.21[bc]	1.60[a]	12.5
Zn, mg/day									
Requirement	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	
Intake - silage	15.7[a]	16.9[a]	17.5[a]	16.5[a]	10.3[b]	9.0[b]	8.0[b]	9.8[b]	18.7
Intake - min. mix.	4.1	26.0	48.1	47.4	4.1	26.0	48.1	47.4	
Total	19.8[e]	42.9[c]	65.6[a]	63.9[a]	14.4[f]	36.0[d]	56.1[b]	57.2[b]	5.5
Mn, mg/day									
Requirement	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	
Intake - silage	50.7[b]	54.0[b]	56.0[b]	53.0[b]	174.4[a]	154.7[a]	140.5[a]	166.1[a]	17.7
Intake - min. mix.	1.0	2.4	4.0	5.4	1.0	2.4	4.0	5.4	
Total	51.7	56.4	60.0	58.4	175.4	157.1	144.5	171.5	
Cu, mg/day									
Requirement	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	
Intake - silage	3.1[a]	3.3[a]	3.5[a]	3.3[a]	1.6[b]	1.3[b]	1.1[b]	1.5[b]	18.6
Intake - min. mix.	.5	5.9	12.2	9.4	.5	5.9	12.2	9.4	
Total	3.6[b]	9.2[d]	15.7[a]	12.7[b]	2.0[g]	7.2[e]	13.4[b]	10.9[c]	4.7

[1]Values are the means of 3 observations.

[2]Intake of CP includes the protein equivalent of 10 g of urea.

[3]Intake of DM and CP of this treatment is the mean of 2 observations.

[abcdefg]Means on the same row with different superscripts differ (P<.05).

Table 37. Apparent (App.) digestibility of DM (DDM), OM (DOM), energy (DE), CP (DCP), and nitrogen balance (NB) and apparent (App.) absorption (Abs) of ash, Ca, P, Mg, Zn, Mn, and Cu of silages supplemented with 4 levels of mineral elements to sheep in metabolism cages[1]

	Whole plant corn silage			
	0	1	2	3
App. DDM, %	59.7^{a}	60.8^{a}	56.7^{a}	59.8^{a}
App. DOM, %	60.8^{a}	62.3^{a}	58.4^{a}	61.5^{a}
App. DE, %	58.8^{a}	58.9^{a}	55.3^{a}	57.2^{a}
App. DCP, %	60.2^{a}	58.3^{a}	54.1^{a}	60.4^{a}
NB, $g/Kg^{0.75}/day$	$.15^{a}$	$.17^{a}$	$.13^{a}$	$.21^{a}$
App. Abs Ash, %	41.6^{a}	33.9^{bcd}	29.2^{g}	37.0^{abc}
App. Abs Ca, %	-2.4^{c}	15.8^{ab}	20.3^{a}	14.7^{ab}
App. Abs P, %	14.3^{a}	15.2^{a}	16.1^{a}	16.3^{a}
App. Abs Mg, %	58.2^{a}	53.6^{ab}	43.7^{b}	42.9^{b}
App. Abs Zn, %	62.0^{a}	69.5^{a}	51.4^{a}	45.2^{ab}
App. Abs Mn, %	32.9^{a}	30.5^{a}	20.9^{ab}	10.5^{bc}
App. Abs Cu, %	34.6^{a}	36.8^{a}	38.1^{a}	13.1^{ab}

[1]Values are the means of 3 observations.

[2]Values of App. DDM, DOM, DCP, DE, and App. Abs Ash are the means of 2 observations.

[abcd]Means on the same row with different superscripts differ ($P<.05$).

Elephant grass silage				
0	1	2[2]	3	CV
54.4[b]	54.0[b]	55.4[b]	52.8[b]	4.1
55.9[b]	56.1[b]	57.4[b]	55.5[b]	4.0
53.4[b]	52.4[b]	52.9[b]	54.9[b]	6.7
55.4[a]	56.3[a]	57.7[a]	56.6[a]	53.
.02[b]	.03[b]	.03[b]	.02[b]	43.8
38.7[ab]	34.5[bcd]	35.9[abc]	31.2[cd]	9.1
3.3[bc]	4.3[abc]	13.3[abc]	6.2[abc]	85.3
2.9[a]	4.3[a]	12.5[a]	3.0[a]	67.7
28.1[c]	26.2[c]	29.9[c]	22.5[c]	15.9
-33.0[d]	14.7[c]	20.6[bc]	10.3[c]	52.9
0.4[c]	3.7[c]	-2.3[c]	-17.1[d]	76.6
-94.4[d]	-5.6[bc]	12.1[ab]	-20.6[c]	973.8

Table 38. Calculated true absorption of Ca (TABSCa), P (TABSP), Mg (TABSMg), Zn (TABSZn), and Cu[1] (TABSCu) of silages supplemented with 4 levels of mineral elements[1]

	Whole plant corn silage				Elephant grass silage				
	0	1	2	3	0	1	2	3	CV
TABSCa	65.5^{a}	47.6^{b}	39.6^{b}	24.2^{cd}	28.6^{c}	22.7^{cd}	28.1^{c}	14.4^{d}	18.0
TABSP	92.1^{a}	84.5^{ab}	78.3^{bc}	70.9^{c}	73.8^{bc}	67.4^{cd}	74.0^{bc}	59.4^{d}	7.3
TABSMg	67.6^{a}	62.2^{a}	51.9^{b}	50.3^{b}	34.9^{c}	33.3^{c}	37.9^{c}	29.1^{c}	11.8
TABSZn	75.1^{a}	75.4^{a}	55.3^{a}	48.8^{ab}	-16.4^{c}	21.2^{b}	24.9^{b}	21.5^{b}	38.2
TABSCu	38.5^{a}	38.2^{a}	38.9^{a}	14.0^{ab}	-88.2^{d}	-3.9^{bc}	13.1^{ab}	-19.4^{bc}	448.8

[1]Values are the means of 3 observations.

[abcd]Means on the same row with different superscripts differ ($P<.05$).

Mn, and Cu) from silage was not affected (P>.50) by the level of mineral elements in the mineral mixture fed to sheep. However, the type of silage did affect (P<.05) the intake of DM, Ca, Zn, Mn, and Cu from the roughage. The apparent DDM, DOM, and DE was not changed (P<.70) by increasing the level of Ca, P, Zn, and Cu in the diet, but was affected by the type of silage (P<.05). Nitrogen balance, expressed in $g/kg^{.75}/day$ was higher (P<.05) in animals fed whole plant corn silage rather than grass silage.

The apparent absorption of P, Mg, Zn, Mn, and Cu were significantly different (P<.01) between the 2 types of silages. The level of mineral elements fed to sheep also affected (P< .05) the apparent absorption of Ca, Zn, Mn, and Cu.

The calculated true absorption of Ca, P, Mg, Zn, and Cu were changed (P<.001) by the type of silage and that of Ca, P, Mg, and Cu was also affected by the level of these mineral elements in the diet.

The % true absorption of Ca and P from these silages was within the range of availability of these elements in most feedstuffs, which was accepted by the committees responsible for preparing the mineral requirement standards of Beef cattle (NRC, 1976), Dairy cattle (NRC, 1978), and livestock (ARC, 1980), as shown in Table 2.

The availability (true absorption) of Mg, Zn, and Cu of whole plant corn silage was higher than the values reported

for most feeds in the literature. The negative true absorption of Zn and Cu of elephant grass silage is not understood. Some unaccounted sources of intake of these elements, such as the drinking water and/or licking the metal feeders and cages, could have occurred. This might have contributed to the higher excretion (corrected for endogenous loss) than intake of these mineral elements. Another possibility would be, if this low-quality, higher fiber tropical forage had caused an increase in endogenous loss (by increasing loss of intestinal cells or secretions) of the mineral elements, it would also partially explain the lower availability of all mineral elements in elephant grass when compared with whole plant corn silage.

GENERAL DISCUSSION AND CONCLUSIONS

The chemical composition of the crop residues found in the current work was generally in agreement with the limited amount of information reported thus far in the literature. The low levels of CP (<5%) and P (<.1%) observed were within the expected range of CP and P content of crop residues. Some trace elements (Zn, Mn, Cu) also might be considered low when compared with the requirement standards for these mineral elements for Beef and Dairy cattle.

Differences between the CP, Ca, and P content of whole plant corn silage grown in Iowa and that grown in Brazil (state of Minas Gerais) is not understood at this time. These lower values might be attributed to the lower native soil fertility and lower fertilization regimen currently practiced together with the heavier rainfall prevalent in that particular state of Brazil as compared with the state of Iowa.

The low content of CP, P, and potentially available energy in crop residues is in contrast to the higher levels observed in immature whole-plant forage feeds. It is important to remember that the forage feeds generally are harvested at a stage of growth where there is a large amount of soluble nutrients. Crop residues generally are parts of mature plants. Most of the soluble nutrients have been translocated to the seeds which are usually harvested for either human consumption or to be fed to livestock.

One of the consequences of the low digestibility of crop residues is their slow rate of passage through the rumen (high mean rumen retention time). Thus, the feed intake of the animals fed crop residues will be low. Fortunately, it is possible to improve the digestibility of crop residues somewhat by chemical treatments, such as using sodium hydroxide (Klopfenstein et al., 1979). In this way, it is possible to maintain a high intake of digestible energy which is necessary if the objective is to use crop residues as a major source of energy for the ruminant animal.

Of the three laboratory techniques utilized in this study, namely (1) *in vitro* inoculation with rumen liquor, (2) cellulase and (3) nylon bag, the latter two proved to be most effective.

In the initial trials with cellulase enzymes, they were not effective in solubilizing DM of forage feeds and crop residues without an initial pretreatment. Although the commercially available cellulase enzymes were crude preparations, having some specificity for digesting cellulose, hemicellulose and proteins (Jones and Hayward, 1973; Jarrige et al., 1970), they by themselves were less effective in solubilizing cell walls than a mixed microbial inoculum of rumen liquor. A pretreatment of feed samples with either neutral-detergent fiber (NDF) solution or pepsin-HCl was necessary in order to make them effective, presumably by exposing the

cell walls such that they could be digested by the cellulase enzymes.

Pretreatment of samples with .2% pepsin-HCl at 40 C, previous to their incubation with cellulase enzymes of American origin resulted in increased disappearance of DM and ash of both alfalfa hay and corn stover samples. These disappearance values were similar in amounts to those obtained with the nylon bag-pepsin technique. Although the pepsin-cellulase technique was effective when a cellulase enzyme from a different chemical company (Brazilian origin) was used, it predicted lower ($P<.05$) digestibility of DM of forages and crop residues than did the nylon bag-pepsin technique, but similar ($P>.05$) disappearance of N from these feedstuffs. The literature revealed no report on the use of the pepsin-cellulase procedure or another similar technique to predict the digestion of CP of feedstuffs. Thus, the pepsin-cellulase procedure seemed to be as good as the nylon-bag-pepsin technique for predicting the whole digestive tract utilization of roughage feed protein, as judged by the agreement between the two procedures.

The nylon bag technique (<u>in situ</u> digestion of feedstuffs in the rumen) offers some advantages over the cellulase or pepsin-cellulase technique. In the former procedure, it is possible to separate the effects of the rumen digestion from that of the whole digestive tract digestion when either one or two digestion phases is used. This does not seem to be the

case with the cellulase technique, at least in the experiments of the current work. The nylon bag technique also is attractive since the rate of digestion in the rumen can be sequentially determined. This can contribute to a better understanding of the limitations and usefulness of each feed as a source of energy, protein, or mineral elements for the ruminant animal.

Inconsistent results have been reported in the literature on the use of the nylon bag technique. Some of the problems have been: sample size in relation to bag size, pore size of the cloth, losses of particulate matter through the cloth mesh as well as entry of rumen contents into the bags.

In the present work, it was observed that repeatable results were comparable to literature values if the sample weight in each case was kept around 20 mg/cm^2 of bag surface area, using either nylon or dacron polyester cloth of at least 30 micron pore size.

The data from the present research suggest that loss of particulate matter should be measured for each kind of cloth and for each sample type used. The extent of loss may vary with the pore size of the cloth and with plant species and/or plant parts, such as leaves, stems, pods, and cobs.

A correction equation, shown in the experimental procedure section, adapted from Playne et al. (1978a) was used in this work. It was understood that it would be better to use

larger pore size cloth and correction factors for the efflux and influx of particulate matter rather than to use a small pore size cloth which seems to restrict digestion, due to the limited influx of digesting agents into the bags (Weakley et al., 1983).

The nylon bag technique is useful for predicting the digestible energy content of feeds or at least that portion of the organic matter which is potentially available for fermentation in the rumen. Another important use of the technique is in the prediction of that portion of dietary protein which will be degraded in the rumen. Protein degradability is estimated from the disappearance of N from bags and from the rate constant for passage of undegraded CP from the rumen.

The results of the CP and P disappearance from the feeds contained in nylon bags seemed to indicate that contamination of the feed residues with microbial matter during digestion had occurred. A similar contamination was reported by Mathers and Aitchison (1981). They infused ^{35}S intraruminally in order to measure the proportion of total protein in the residues in nylon bags which were of microbial origin. Therefore, it would be necessary to measure the contribution of the microbial matter to the total DM present in the residue. This could also be accomplished by measuring the concentration of D-alanine in the residues. Then a correction for the microbial N and major mineral elements in the residue could be made assuming

the microbial matter has 10% N (Hungate, 1966) and a mineral composition similar to that given by Durand and Kawashima (1980). A more accurate approach to the problem would be to determine the concentration of a microbial marker (D-alanine, ^{35}S, ^{33}P) in nonammonia nitrogen (NAN) present in the nylon bag residue and in NAN microbial matter isolated from the rumen digesta. Thus, the proportion of microbial matter in the bag could be related to that outside the bag.

The nylon bag technique is also useful in measuring the solubility (release) of dietary mineral elements upon digestion of feeds in the rumen. According to Field (1981), the extent of release of macro-elements in the digestive tract is a neglected subject. There appears to be only one quantitative study (Playne et al., 1978b) relating the release of minerals in 4 tropical hays during their digestion in the rumen. There is no report of the release of mineral elements from crop residues in the rumen.

Field (1981) stated that availability (absorption) of dietary macroelements is dependent upon two processes; the release and the absorption of soluble element. Thus, absorption (A) would be equal to the fractional absorption (B) of dietary intake (I) which also would be equal to the fractional absorption (C) of the dietary (I) mineral element solubilized in the stomach (rumen) (M_S); $A = BI = CM_SI$.

Field (1981) also mentioned a possible additional release

of Ca in the acid conditions of the abomasum and indicated the methodology needed for such an analysis which I quote:

> Since dietary Ca is released in the abomasum and absorbed in the small intestine, M_S must be measured *in vivo* at the duodenum near to the pylorus or *in vitro* by subjecting feed residues after rumen digestion to simulated abomasal conditions.

However, treatment of feeds with .2% pepsin-HCl prior to the cellulase treatment (Appendix Table A5) or after feeds had been digested in nylon bags in the rumen (Tables 25 and 26) resulted in 97-100% disappearance of the feed Ca originally present. The disagreement between the expected coefficient of absorption of Ca in the system proposed by Field (1981) and that obtained in the present work is not understood at this time. No report is available for direct comparison of the data, using this two-stage nylon bag-pepsin or other technique which uses the pepsin-HCl as one of the digestion stages, for predicting the release of mineral elements from feedstuffs during digestion.

The ruminal digestion phase of the nylon bag technique (*in situ* digestion of feeds in the rumen) showed that Ca, Zn, and Mn disappearance seemed to follow the DM disappearance of the respective feed sample, while that of Mg, P, and K seemed to be related to the water-soluble fraction. Most of the potentially available P, Mg and K were solubilized in the first 6 h in the rumen or by water extraction. Another important factor observed was that small differences among feed samples

were found in the release of Mg and K, thus little benefit would be gained by measuring the release (M_s) of Mg and K for different feedstuffs. This suggests that C must be similar but slightly higher than B.

A larger variation in M_s was observed for Ca and trace elements (Zn and Mn) than for Mg and K. Since lactating cows and pregnant ewes utilize the Ca in low Ca diets with high efficiency (Sykes and Field, 1972, as cited by Field, 1981), it might be postulated that the M_s of Ca for different feedstuffs may approximate the factor for converting net to dietary requirements of this element (Field, 1981).

It would be interesting to know if the results obtained for the trace elements reflect the potentially available Zn and Mn in forage and crop residues or if they are an artifact due to microbial contamination of the residues. Beveridge and Murray (1980) reported that the cell walls of a gram positive bacteria bind a large amount of metals. Wallace (1983) implied that changes in the bacterial population which had a negligible effect on the digestibility of bacterial protein might have a major influence on the availability of trace elements. The data in the current research support in part this hypothesis. It showed that the release of trace elements was lower than that of the DM and CP in a large number of feedstuffs. There was also a large variation among feedstuffs. Therefore, large differences among feedstuffs in

potential availability of trace elements (Zn and Mn) in the rumen seemed to exist.

It can be said that not only the content (total amount present) but also the potential availability of CP, P and some trace elements contained in crop residues probably are lower than that of the immature forage feeds. Thus, these differences in availability might be important to have in mind when using the mineral requirement standards of cattle in feeding programs. It is also important to relate these values to the average value used by the committees responsible for preparing these standards, to convert from net to dietary requirements of these elements.

Before concluding this general discussion, a few remarks should be made concerning the most desirable laboratory technique to employ in measuring the potential utilization of crop residues and forages. Also, the degree of similarity one might expect from these laboratory procedures as compared with in vivo measurements of digestibility in ruminant animals. An answer to both questions can be obtained from Table 39. This table presents the summary of the DM disappearance of 3 crop residues measured either in vivo or by 3 different laboratory techniques. One can conclude from the summary of the results presented in Table 39 that the nylon bag technique gave the most consistent results for the prediction of the potential utilization of the 3 crop residues as compared with

Table 39. DM disappearance of crop residues measured either *in vivo* or by 3 different laboratory techniques

Crop residue	Corn stover and corn stover silage	Soybean stover	Oat straw
In vivo[a]	49	41	43
Nylon bag[b]	45	37	40
Nylon bag-pepsin[c]	47	39	51
Pepsin-cellulase[d]	34	36	35

[a]Average of the *in vivo* DMD reported by the following authors: Corn stover and corn stover silage - (1) Colenbrander et al. (1973), (2) Elhag (1976), (3) Klopfenstein (1978), (4) Vandersall et al. (1972); Soybean stover - (1) Elhag (1976), (2) Gupta et al. (1973); Oat straw - (1) Elhag (1976).

[b]Samples digested in nylon bags in the rumen for 48 h, corrected for losses of particulate matter through the pores of the cloth.

[c]Samples digested in nylon bags in the rumen for 48 h and incubated in pepsin-HCl for 24 h at 39 C.

[d]Samples incubated in pepsin-HCl for 24 h at 39 C and then incubated with 6.25 g/l of a Brazilian cellulase enzyme in pH 4.8 buffer.

the *in vivo* digestibility value. This simple one-stage digestion procedure of feedstuffs (crop residues) in the rumen predicted that 90% of the *in vivo* digestible DM would be digested after 48 h of rumen exposure. This conclusion is in agreement with the results of Armstrong and Beever (1969) who reported that only 10% of the carbohydrates of feeds were digested in the intestine of adult ruminants.

The pepsin-cellulase technique is an alternate procedure which can be used if (1) the appropriate cellulase enzyme is commercially available and (2) the maintenance of 2 or 3 rumen fistulated bovine animals needed in the nylon bag technique is not possible or desirable.

SUMMARY

The purpose of the present research was to determine the N and mineral composition of selected forages (alfalfa, canarygrass, bromegrass, fescue, whole plant corn silage, elephantgrass silage), crop residues (corn, oat, soybean), and crop residue plant parts, as well as to evaluate the potential utilization of DM, N, and some useful mineral elements by laboratory techniques.

The highest concentrations of mineral elements, determined by the dry ash method, were observed when forage feeds and crop residues were ashed at 550 C for 1.5 h.

In general, crop residues were low in CP (<5%), digestibility of DM (<50%), P (<.1%), and perhaps some trace elements, such as Zn (<25 ppm), Mn (< 40 ppm) and Cu (<8 ppm).

Whole plant corn silage grown in Brazil (state of Minas Gerais) was lower in CP, Ca, and P than that grown in the state of Iowa. Lower soil fertility and fertilization regimen, as well as heavier rainfall prevalent in Brazil compared with Iowa might have been important factors affecting silage composition.

The low content of CP, P, and potentially available energy in crop residues is in contrast to the higher levels observed in immature whole-plant forage feeds.

In the initial trials with cellulase enzymes, they were not effective in solubilizing DM of forage feeds and crop

residues without an initial pretreatment. A pretreatment with pepsin-HCl seemed necessary in order to make them effective, presumably by exposing the cell walls such that they could be digested by the cellulase enzymes.

Pretreatment of samples with .2% pepsin-HCl at 40 C, previous to their incubation with cellulase enzymes, resulted in increased ($P<.01$) disappearance of DM of alfalfa hay and corn stover. These values were similar to those obtained with the nylon bag-pepsin technique. However, when another cellulase enzyme (Brazilian) was used, it predicted lower ($P<.05$) digestibility of DM of forages and crop residues than did the nylon bag-pepsin technique, but similar ($P>.05$) disappearance of N from these feedstuffs.

Inclusion of a 2nd stage of digestion with pepsin-HCl after the fermentation in nylon bags in the rumen resulted in substantially higher ($P<.01$) disappearance of CP and mineral elements (Ca, P, Mg, K, Zn, Mn, and Cu). In fact, the disappearance of almost all mineral elements reached values above 90% of the initial content present in the bag, in all feedstuffs studied, even after 24 h of digestion. Similar results were obtained when a pepsin-cellulase procedure was used. Therefore, any technique that includes incubation in .2% pepsin-HCl may not be useful for studying the potentially available mineral elements in feedstuffs.

The nylon bag technique was found to give repeatable

results that were comparable to literature values if the sample weight was kept around 20 mg/cm^2 of bag surface area, using either nylon or dacron polyester cloth of at least 30 micron pore size.

Losses of particulate matter through bag cloth ranged from 2-14%, depending on the type of feed (roughage). A correction factor for this efflux of particulate matter was used instead of a small pore size cloth which seems to restrict digestion.

Disappearance of DM and CP of crop residues from nylon bags in the rumen was lower than 45% and 55%, respectively. However, the disappearance of DM of forage feeds, and soybean leaves and pods ranged from 58-67% while that of the CP in these feeds was around 80%.

Disappearance of Ca, Zn, and Mn seemed to follow the DM disappearance of the respective sample, while that of P, K, and Mg was readily released from bags. A significant linear and quadratic effect ($P<.01$) of time on the disappearance of DM, CP, Ca, Zn, and Mn from feedstuffs contained in nylon bags was observed.

Crop residues with low initial levels of CP, P and trace elements, sometimes showed negative disappearance of these constituents of the total DM from nylon bags in the rumen, indicating a probable contamination with adherent bacteria attached to feed particles.

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APPENDIX

Table A1. Content of ash in the residue from solubilization for 4 different enzymes and 2 buffers[a]

	pH 7.0 buffer			pH 4.8 buffer		
	Buffer only	Protease WT	Amylase WT	Buffer only	Cellulase WT	Cellulase (*T. viride*)
Alfalfa hay	5.99	5.72	3.91	2.63	1.75	2.02
Corn stover	14.52	12.71	13.25	10.80	11.60	12.64
Dry poultry waste	48.80	52.68	50.37	33.77	38.79	37.59
Oat straw	6.46	6.66	6.77	6.39	6.75	7.10
Soybean meal	2.76	4.15	2.91	1.75	1.55	1.69

[a]Values are the mean of 2 observations.

Table A2. Content of CP and ash in the residue of alfalfa hay and corn stover samples pretreated with either HCl or pepsin-HCl and treated with increasing levels of cellulase[a]

Pretreatment	Enzyme			
	Buffer only	6.25	12.50	25.00
	Crude protein, % DM			
Alfalfa hay				
.1 N HCl	23.15	24.63	22.77	20.18
.2% pepsin-HCl	8.83	12.88	12.76	10.98
Corn stover				
.1 N HCl	3.27	3.27	3.38	3.00
.2% pepsin-HCl	2.29	2.74	2.63	2.33
	Ash, % DM			
Alfalfa hay				
.1 N HCl	1.27	1.01	.70	.77
.2% pepsin-HCl	1.50	1.14	.89	.72
Corn stover				
.1 N HCl	10.40	13.22	14.62	13.35
.2% pepsin-HCl	10.43	13.94	15.08	13.14

[a]Values are the mean of 3 observations.

Table A3. Content of Ca, P, Mg, and K in the residue of samples pretreated with either HCl or pepsin-HCl and treated with increasing levels of cellulase[a]

Pretreatment	Cellulase enzyme, g/l			
	Buffer only	6.25	12.50	25.00
	Ca, % DM			
Alfalfa hay				
.1 N HCl	.036	.023	.011	.013
.2% pepsin-HCl	.059	.020	.019	.014
Corn stover				
.1 N HCl	.009	.010	.006	.009
.2% pepsin-HCl	.006	.006	.008	.006
	P, % DM			
Alfalfa hay				
.1 N HCl	.039	.041	.037	.037
.2% pepsin-HCl	.034	.034	.040	.032
Corn stover				
.1 N HCl	.015	.013	.012	.013
.2% pepsin-HCl	.013	.015	-	.020
	Mg, % DM			
Alfalfa hay				
.1 N HCl	.001	.003	.006	.009
.2% pepsin-HCl	.001	.004	.006	.009
Corn stover				
.1 N HCl	.003	.005	.007	.009
.2% pepsin-HCl	.004	.006	.008	.010
	K, % DM			
Alfalfa hay				
.1 N HCl	.022	.010	.003	.005
.2% pepsin-HCl	.012	.006	.005	.005
Corn stover				
.1 N HCl	.026	.045	.032	.032
.2% pepsin-HCl	.033	.036	.035	.033

[a]Values are the mean of 3 observations.

Table A4. Solubilization of DM, CP, and ash of smooth bromegrass sample and the content of CP and ash in the residue of incubation with pepsin-HCl (24 h at 40 C), followed by increasing levels of cellulase enzyme (48 h at 40 C)[1,2,3]

	Weight of enzyme, g/l				
	Buffer only	3.0	6.0	12.0	CV
DM solubilized, %	39.52[c]	50.31[b]	53.39[a]	54.26[a]	2.34
CP solubilized, % DM	70.61[d]	77.66[c]	83.27[a]	82.23[b]	.53
Ash solubilized, % DM	71.17[c]	74.00[b]	76.23[a]	76.65[a]	.79
Content of CP in the residue, % DM	8.02	7.43	5.87	6.41	
Content of ash in the residue, % DM	5.72	6.28	6.13	6.13	

[1]Values are the mean of 3 observations.

[2]Enzyme dissolved in 300 ml of citrate-phosphate buffer pH 4.8.

[3]3 g sample.

[abcd]Means in the same row with different superscripts differ (P<.05).

Table A5. Content of CP, ash, Ca, P, Mg, K, Zn, Mn, and Cu in the residue of 17 different samples solubilized by the pepsin-cellulase technique[a,b]

Samples	CP %	Ash %	Ca ppm	P ppm	Mg ppm	K ppm	Zn ppm	Mn ppm	Cu ppm
Soybean stover	1.9	1.3	57	213	10	61	1.6	-[c]	.7
Soybean stalks	1.8	1.4	49	243	8	83	3.1	-	-
Soybean leaves	9.4	31.0	360	870	259	611	13.3	14.0	2.5
Soybean pods	2.9	1.2	111	395	15	90	13.3	-	.8
Corn stover	2.4	9.3	36	560	30	140	3.1	-	1.1
Corn stover	2.0	3.6	24	440	19	79	2.3	.9	.7
Corn stover silage	2.8	6.8	21	363	31	144	3.1	1.3	1.5
Corn husks	1.5	2.2	21	453	6	44	1.7	1.1	-
Cornstalks	2.1	2.6	25	758	12	44	3.2	1.3	1.1
Corn leaves	3.4	8.5	47	807	22	72	3.4	1.3	1.1
Oat straw	1.9	6.8	21	423	8	43	4.2	-	.4
Alfalfa hay	6.1	2.5	369	700	9	106	5.6	-	2.3
Alfalfa hay	8.3	.6	252	320	15	87	4.8	-	4.1
Alfalfa, 2nd cut	7.7	.8	150	485	15	88	5.9	-	2.0
Reed canarygrass	7.6	7.3	40	920	29	166	4.8	1.1	4.6
Smooth bromegrass	7.6	11.2	36	723	25	74	5.3	-	3.8
Tall fescue	6.2	6.8	46	633	13	64	2.2	-	2.8

[a]Mean of two trials with 2 observations each.

[b]Dry matter basis.

[c]- indicates not detectable.

Table A6. Content of CP, ash, Ca, P, Mg, K, Zn, Mn, and Cu in the residue of 17 different samples digested by the nylon bag technique[a,b]

Sample	CP %	Ash %	Ca ppm	P ppm	Mg ppm	K ppm	Zn ppm	Mn ppm	Cu ppm
Soybean stover	2.4	1.3	44	268	12	32	2.9	-[c]	2.8
Soybean stalks	2.3	1.7	28	274	10	50	4.3	-	2.9
Soybean leaves	8.7	39.2	137	622	266	529	13.5	25.6	2.4
Soybean pods	2.6	1.2	153	272	17	32	4.6	-	1.7
Corn stover	2.6	9.4	28	400	45	115	3.5	2.5	7.2
Corn stover	3.0	5.4	59	397	14	48	5.4	2.1	3.6
Corn stover silage	3.7	7.8	26	448	47	113	4.9	2.0	3.7
Corn husks	2.1	2.1	43	282	12	25	5.1	1.5	2.6
Cornstalks	3.2	2.9	35	450	10	46	2.6	3.6	3.7
Corn leaves	4.7	8.9	30	438	14	55	4.7	2.0	4.1
Oat straw	3.1	7.9	33	423	7	70	2.8	-	3.4
Alfalfa hay	4.9	2.5	260	488	10	42	4.6	-	6.4
Alfalfa hay	6.4	.7	429	520	8	45	12.0	-	3.2
Alfalfa, 2nd cut	6.1	1.1	291	443	14	56	4.7	-	4.4
Reed canarygrass	6.8	9.8	41	638	17	98	7.7	1.4	7.0
Smooth bromegrass	9.6	12.9	38	560	18	70	4.6	-	7.9
Tall fescue	6.2	8.0	54	572	11	60	5.4	-	6.1

[a]Mean of 3 trials.

[b]Dry matter basis.

[c]- indicates not detectable.

Table A7. Disappearance of DM, OM, CP, ash and P in a preliminary test of digestion of alfalfa hay and corn stover in nylon bags in the rumen for 48 h, followed by a 24 h incubation in pepsin-HCl[a]

Sample	Alfalfa hay	Corn stover
Disappearance of DM, %	66.80	52.00
Disappearance of OM, % DM	64.25	52.02
Disappearance of CP, % DM	87.13	49.61
Disappearance of ash, % DM	95.59	51.91
Disappearance of P, % DM	90.16	22.00

[a]Mean of 3 observations.

Table A8. Content of CP, ash, Ca, P, Mg, K, and Zn in the residue of samples digested in nylon bags in the rumen for 12, 24, 48, and 72 h, followed by a 24 h incubation in pepsin-HCl[a]

Sample	Rumen digestion time, h			
	12	24	48	72
	Alfalfa hay			
Crude protein	24.02	14.04	10.40	11.29
Ash, % DM	3.43	1.20	.96	1.51
Calcium, % DM	.055	.030	.130	.422
Phosphorus, % DM	.14	.17	.11	.09
Magnesium, ppm DM	33	25	30	37
Potassium, ppm DM	10	10	10	23
Zinc, ppm DM	10.8	12.2	6.2	9.6
	Corn stover			
Crude protein, % DM	6.36	7.20	4.57	4.91
Ash, % DM	10.47	11.07	11.39	14.26
Calcium, % DM	.028	.013	.008	.008
Phosphorus, % DM	.12	.17	.08	.11
Magnesium, ppm DM	83	70	63	83
Potassium, ppm DM	43	47	30	33
Zinc, ppm DM	9.4	14.4	6.7	11.4

[a]Mean of 2 observations.

Table A9. Content of CP, ash, Ca, P, Mg, Mn, and Cu in the residue of samples digested in nylon bags in the rumen for 24, 48, and 72 h, followed by a 24 h incubation in pepsin-HCl in 3 trials conducted in Brazil[a]

Sample	Rumen digestion time, h		
	24	48	72
	Alfalfa hay		
Crude protein, % DM	7.37	6.69	6.67
Ash, % DM	.97	1.36	1.23
Calcium, ppm DM	664	652	468
Phosphorus, % DM	.066	.059	.051
Magnesium, ppm DM	21	25	25
Manganese, ppm DM	-[b]	-	-
Copper	5	5	5
	Corn stover		
Crude protein, % DM	3.08	4.42	3.69
Ash, % DM	10.41	11.25	12.63
Calcium, ppm DM	51	73	60
Phosphorus, % M	.047	.078	.091
Magnesium, ppm DM	68	68	58
Manganese, ppm DM	4	4	4
Copper, ppm DM	2	3	4

[a]Mean of 3 trials.

[b]- indicates not detectable.

Table A10. Content of DP, ash, Ca, P, Mg, K, Zn, Mn, and Cu in the residue from digestion in nylon bags for 24, 48, and 72 h, followed or not by a 24 h incubation with pepsin-HCl[a]

	Rumen digestion time, h					
	24		48		72	
	--Pepsin-HCl--					
Sample	+	-	+	-	+	-
Alfalfa hay						
Crude protein, %	7.0	13.9	6.1	10.7	6.7	10.4
Ash, %	.7	2.9	1.5	3.8	1.5	4.1
Macromineral elements, expressed in:	ppm	%	ppm	%	ppm	%
Calcium	476	.94	694	1.02	1052	1.11
Phosphorus	440	.07	410	.07	480	.08
Magnesium	12	.11	8	.07	12	.07
Potassium	34	.04	24	.03	37	.03
Micromineral elements, expressed in ppm						
Zinc	-[b]	97	-	33	-	46
Manganese	-	83	-	56	-	56
Copper	6	10	5	8	5	10
Corn stover						
Crude protein, %	2.6	4.1	4.6	6.1	3.8	5 5.9
Ash, %	9.8	10.3	11.3	11.9	13.6	14.5
Macromineral elements, expressed in:	ppm	%	ppm	%	ppm	%
Calcium	45	.28	36	.30	84	.32
Phosphorus	340	.03	700	. 2	970	.15
Magnesium	63	-	53		61	
Potassium	165	.11	145	.12	170	.11
Micromineral elements, expressed in ppm						
Zinc	-	72	-	42	-	43
Manganese	4	54	2	63	3	82
Copper	2	4	3	4	4	5

[a]Dry matter basis.

[b]-, not detectable.

Table A11. Content of CP, ash, Ca, P, Mg, K, Zn, Mn, and Cu in the residue of digestion in nylon bags for 48 h, followed or not by a 24 h inducation with pepsin-HCl[a]

	Corn silage				Elephant grass silage			
	Trial I		Trial II		Trial I		Trial II	
	--Pepsin-HCl--							
Sample	+	-	+	-	+	-	+	-
Crude protein, %	2.8	3.8	3.4	4.6	2.9	3.7	3.1	4.1
Ash, %	3.9	4.3	3.8	5.2	6.3	7.0	6.9	7.6
Macromineral elements, expressed in:	ppm	%	ppm	%	ppm	%	ppm	%
Calcium	-[b]	.18	-	.16	5	.24	12	.32
Phosphorus	225	.06	385	.06	360	.07	415	.08
Magnesium	6	.07	8	.06	2	.04	3	.03
Potassium	34	.02	47	.02	20	.02	20	.04
Micromineral elements, expressed in ppm								
Zinc	3.8	56.0	2.8	21.9	2.4	56.6	1.0	18.1
Manganese	-	14.0	-	18.3	-	37.0	-	55.4
Copper	4.0	5.5	4.3	4.6	3.6	5.2	2.3	4.5

[a]Dry matter basis.

[b]-, not detectable.

Table A12. Content of CP, ash, Ca, P, and Mg and Zn in the residue of samples contained in nylon bags extracted with water for 8 h in a shaker

	Trials 1, 2, and 3[a]		Trial 5[b]		
Sample	Alfalfa hay	Corn stover	Alfalfa hay	Corn stover silage	Corn cobs
Crude protein, % DM	20.8	3.0	21.2	3.0	1.6
Ash, % DM	3.4	7.8	4.2	5.0	.5
Calcium, % DM	1.15	.41	1.31	.22	.02
Phosphorus, % DM	.06	.02	.041	.014	.006
Magnesium, % DM	.09	.13	.116	.047	.030
Zinc, ppm DM			33.3	14.7	17.8

[a]Values are the means of 3 trials with 2 observations each.

[b]Values are the means of 4 observations.

Table A13. Content of CP, ash, Ca, P, Mg, K, and Mn in the residue of samples enclosed in nylon bags extracted with water for 8 h in a shaker, trial 4[a]

Sample	CP	Ash	Ca	P	Mg	K	Mn
	----------% DM----------					---ppm DM----	
Soybean stover	2.8	2.4	.54	.02	.14	46	11.8
Soybean stalks	2.2	2.3	.49	.01	.10	66	8.0
Soybean leaves	9.6	28.7	2.04	.07	.17	683	217.4
Soybean pods	4.4	2.9	1.00	.03	.17	118	10.2
Corn stover	2.9	4.2	.18	.02	.08	102	16.0
Corn stover silage	3.3	7.0	.21	.04	.07	181	15.4
Corn husks	1.6	2.0	.14	.01	.10	170	33.5
Cornstalks	2.5	2.5	.16	.02	.07	409	24.3
Corn leaves	3.3	7.3	.41	.03	.14	103	69.2
Oat straw	2.3	6.4	.24	.03	.07	221	15.9
Alfalfa hay	13.3	3.7	1.13	.06	.10	146	23.4
Alfalfa, 2nd cut	18.6	3.1	1.32	.06	.09	144	19.6
Reed canarygrass	18.0	5.4	.21	.06	.08	166	35.1
Smooth bromegrass	14.6	8.7	.46	.09	.09	164	50.0
Tall fescue	12.7	5.3	.31	.05	.09	136	30.0

[a]Values are the means of 2 observations.

Table A14. Content of CP, ash, Ca, P, Mg, and Mn in the residue of samples digested in nylon bags in the rumen of fistulated animals[a]

Sample	CP, %				Ash, %				Ca, %			
	24	48	72	96	24	48	72	96	24	48	72	96
	Digestion time, h											
SB stover	2.6	2.7	2.6	3.1	1.9	1.9	1.7	1.7	.39	.30	.35	.31
SB stalks	2.6	2.7	2.5		1.8	1.8	1.6		.32	.21	.18	
SB leaves	9.9	9.5	9.5	9.6	30.2	34.1	34.6	33.7	1.24	1.00	.65	.82
SB pods	4.0	3.0	2.9	3.2	2.1	2.0	1.6	2.0	.77	.58	.56	.57
Corn stover	3.8	3.6			4.4	5.0			.15	.17		
Corn stover silage	4.0	3.9			6.6	7.4			.20	.16		
Corn husks	2.6	3.1			2.3	2.7			.20	.16		
Cornstalks		4.3				3.3				.18		
Corn leaves	5.0	4.9			8.2	9.2			.28	.23		
Oat straw	3.2	3.7			6.7	8.0			.22	.18		
Alfalfa hay	8.5	5.8	4.9	6.1	3.5	4.0	4.0	4.1	.68	.58	.69	.86
Alfalfa, 2nd cut	11.2	7.9	7.5	8.6	2.8	3.0	2.6	2.7	1.04	1.02	1.08	.95
Reed canary-grass	12.3	8.7	8.4	9.3	7.9	9.3	9.6	9.7	.31	.31	.35	.46
Smooth brom-grass	16.7	13.0			10.8	13.6			.49	.37		
Tall fescue	10.3	7.7	7.6	9.0	6.3	9.2	9.2	10.1	.31	.32	.32	.41

[a]Values are the means of 4 observations.

Table A14. (Continued)

Sample	P, %				Mg, %				Mn, ppm			
	24	48	72	96	24	48	72	96	24	48	72	96
	Digestion time, h											
SB stover	.03	.03	.04	.04	.07	.06	.07	.06	19.1	15.1	16.9	17.5
SB stalks	.03	.03	.03		.05	.04	.05		19.0	15.5	16.0	
SB leaves	.09	.10	.06	.09	.16	.15	.13	.15	190.8	216.5	139.6	149.0
SB pods	.03	.02	.03	.05	.08	.04	.04	.05	26.5	15.7	16.5	17.3
Corn stover	.04	.05			.04	.04			14.7	14.8		
Corn stover silage	.04	.06			.05	.04			24.1	22.7		
Corn husks	.04	.05			.05	.04			17.7	17.3		
Cornstalks		.06				.04				17.9		
Corn leaves	.05	.06			.05	.05			27.7	23.0		
Oat straw	.04	.06			.04	.03			20.0	15.8		
Alfalfa hay	.07	.07	.08	.13	.09	.05	.06	.07	46.6	42.7	42.4	43.3
Alfalfa, 2nd cut	.07	.07	.07	.08	.08	.06	.07	.08	57.9	62.6	58.1	48.6
Reed canary-grass	.06	.06	.07	.08	.06	.05	.07	.06	28.9	26.5	32.3	29.6
Smooth brome-grass	.10	.09			.07	.07			52.5	47.7		
Tall fescue	.06	.07	.06	.08	.05	.06	.06	.08	23.9	27.9	28.4	30.7

Table A15. Content of CP, ash, Ca, P, Mg, and Zn in the residue of either intact samples digested in the rumen, or prewashed with water previous to the digestion in the rumen of fistulated animals, during either 6, 12, or 24 h[a]

				CP	Ash	Ca	P	Mg	Zn
				% DM					ppm DM
Alfalfa hay	Nylon cloth	Intact	6	21.7	5.3	1.57	.13	.12	34.2
			12	16.9	5.4	1.30	.15	.08	38.7
			24	14.2	6.1	1.33	.11	.07	43.3
	Dacron cloth	Intact	6	21.9	5.0	1.43	.11	.10	34.7
			12	16.9	5.4	1.31	.15	.08	36.5
			24	13.8	6.6	1.36	.17	.08	41.0
	Dacron cloth	Prewashed	6	21.3	4.7	1.37	.059	.116	41.4
			12	16.9	5.2	1.25	.110	.071	38.8
			24	14.2	6.1	1.27	.146	.064	48.8
Corn stover silage	Dacron cloth	Intact	6	4.5	5.9	.42	.061	.055	14.9
			12	5.8	6.6	.40	.093	.055	18.9
			24	7.3	7.6	.40	.144	.059	26.1
	Dacron cloth	Prewashed	6	4.4	5.4	.40	.052	.053	17.2
			12	5.2	5.9	.37	.082	.050	19.6
			24	7.1	7.3	.41	.147	.060	33.1
Corn cobs	Dacron cloth	Intact	6	2.5	1.2	.17	.037	.030	7.4
			12	4.9	1.6	.19	.105	.037	11.4
			24	7.9	2.1	.27	.210	.052	19.1

[a]Values are the means of 9 observations.

Table A16. Disappearance of DM (DDM), CP (DCP), ash (DAsh), Ca (DCa), P (DP), Mg (DMg) and Mn (DMn) from samples contained in nylon bags digested in the rumen of fistulated animals during either 24, 48, 72 or 96 h[1]

		Alfalfa		Reed canary-grass	Tall fescue	Soybean			Mean	CV
		Hay	2nd cut			Stover	Pods	Leaves		
DDM	24	49.5	58.9	51.3	48.2	29.1	47.8	50.0	47.6^{d}	5.3
	48	59.1	67.2	62.9	63.4	36.8	60.1	58.5	58.3^{c}	
	72	63.9	69.8	67.0	66.9	39.2	65.5	61.3	61.9^{b}	
	96	64.9	69.4	67.8	71.2	40.1	67.8	61.4	63.1^{a}	
DCP	24	69.7	75.5	71.6	65.2	51.7	60.0	51.2	63.3^{c}	2.7
	48	83.2	86.2	84.6	81.6	55.4	76.7	61.0	75.4^{b}	
	72	87.5	88.0	86.8	83.5	59.2	80.8	63.5	78.4^{a}	
	96	84.7	86.1	85.7	83.2	50.8	79.9	63.3	76.1^{b}	
DAsh	24	78.5	87.1	68.2	70.5	64.4	88.1	50.1	72.4^{c}	2.8
	48	80.1	89.6	71.3	69.8	67.9	91.3	53.3	74.7^{b}	
	72	82.5	91.5	73.6	72.6	72.8	93.8	55.8	77.6^{a}	
	96	82.5	91.0	74.1	73.8	72.6	93.1	57.0	77.5^{a}	

[1]Values are the means of 8 observations.

[abcd]Means in the same column, within each type of analysis, with different superscripts differ ($P<.05$).

Table A16. (Continued)

		Alfalfa		Reed canary-grass	Tall fescue	Soybean			Mean	CV
		Hay	2nd cut			Stover	Pods	Leaves		
DCa	24	75.6	64.4	63.2	67.9	68.9	69.1	78.8	69.8[c]	2.5
	48	83.2	72.1	72.0	76.6	78.7	82.2	85.8	78.6[b]	
	72	82.3	72.9	71.8	78.8	76.1	85.1	91.4	79.8[a]	
	96	78.6	75.8	63.9	76.4	79.1	85.9	89.2	78.5[b]	
DP	24	86.9	89.8	89.9	89.6	64.6	82.6	62.5	80.8[d]	1.5
	48	89.4	92.4	92.3	91.5	68.4	91.1	65.4	84.3[b]	
	72	89.3	93.0	92.0	93.4	59.5	88.5	80.6	85.0[a]	
	96	83.1	91.9	91.1	92.3	60.1	82.1	71.0	81.5[c]	
DMg	24	85.8	84.1	89.6	92.2	91.1	94.1	86.2	89.0[b]	.9
	48	93.2	91.1	93.4	93.3	93.2	97.6	89.3	93.0[a]	
	72	93.2	90.4	91.8	94.0	92.4	98.1	91.3	93.0[a]	
	96	92.3	88.9	93.1	93.3	93.6	97.7	90.0	92.8[a]	
DMn	24	32.3	16.8	77.9	77/5	32.3	41.4	49.6	47.4[d]	5.4
	48	49.5	28.2	84.6	81.4	52.3	73.5	52.5	60.5[c]	
	72	55.8	38.7	83.3	82.9	48.7	75.9	71.5	64.9[b]	
	96	56.0	48.1	85.0	83.9	47.6	75.7	69.6	66.9[a]	

Table A17. Disappearance of DM (DDM), CP (DCP), ash (DAsh), Ca (DCa), P (DP), Mg (DMg), and Zn (DZn) from samples contained in nylon bags digested in the rumen of fistulated animals during either 6, 12, or 24 h[1]

		Alfalfa hay	Corn stover silage	Corn cobs	Mean	CV
DDM	6	39.7^{c}	7.2^{c}	4.7^{c}	17.2	10.4
	12	60.7^{b}	18.3^{b}	18.5^{b}	32.5	
	24	70.1^{a}	33.7^{a}	38.6^{a}	47.3	
DCP	6	43.6^{c}	20.5^{a}	-14.4^{a}	16.6	1180.4
	12	71.5^{b}	10.3^{b}	-92.5^{b}	-3.6	
	24	82.6^{a}	8.7^{b}	-138.8^{c}	-18.4	
DAsh	6	68.7^{a}	40.8^{a}	35.7^{a}	48.4	16.0
	12	77.9^{a}	41.7^{a}	30.3^{a}	50.0	
	24	79.3^{a}	45.1^{a}	23.1^{b}	48.6	
DCa	6	41.4^{b}	7.2^{b}	-599.8^{a}	-183.7	20.7
	12	65.1^{ab}	23.1^{ab}	-588.4^{a}	-166.7	
	24	72.6^{a}	37.3^{a}	-608.6^{a}	-175.3	
DP	6	74.3^{a}	50.2^{a}	2.1^{a}	42.5	349.0
	12	78.2^{a}	33.3^{b}	-133.8^{b}	-7.5	
	24	80.6^{a}	15.4^{c}	-260.4	-59.0	
DMg	6	82.3^{b}	75.3^{c}	17.7^{a}	58.4	6.1
	12	91.5^{a}	78.5^{b}	13.8^{b}	61.2	
	24	93.6^{a}	81.1^{a}	9.6^{c}	60.4	
DZn	6	38.0^{c}	9.4^{a}	63.3^{a}	36.9	25.6
	12	57.4^{b}	$-.5^{b}$	52.0^{b}	36.3	
	24	64.1^{a}	-12.7^{c}	39.3^{c}	30.4	

[1]Values are the means of 18 observations.

[abc]Means in the same column, within each type of analysis, with different superscripts differ ($P<.01$).

Table A18. Content of CP, ash, Ca, P, Mg, Zn, Mn, and Cu in the feces of sheep fed silages supplemented with 4 levels of mineral elements[a]

Sample	Mineral element mixture 0	1	2	3
Corn silage				
Crude protein, % DM	9.2	9.7	9.6	9.2
Ash, % DM	7.3	8.5	9.3	10.7
Calcium, % DM	.29	.48	.64	1.71
Phosphorus, % DM	.38	.55	.67	.97
Magnesium, % DM	.16	.19	.21	.23
Elephant grass silage				
Crude protein, % DM	7.2	7.3	7.2	7.0
Ash, % DM	11.8	12.9	14.3	16.1
Calcium, % DM	.63	.91	1.18	1.93
Phosphorus, % DM	.40	.61	.80	1.06
Magnesium, % DM	.33	.34	.37	.37

[a]Values are the means of 3 observations.

Table B1. Analysis of variance of ash, Ca, K, Zn and Cu content of samples ashed at 3 different temperatures and 2 lengths of time of ashing

Source of variation	DF	Mean square	F	Prob>F
Ash				
Temperature	2	12.513	23.33	.001
Time	1	3.325	6.20	.016
Sample	4	3920.366	7310.71	.001
Temperature x time	2	.637	1.19	.312
Temperature x sample	8	10.621	19.81	.001
Time x sample	4	1.979	3.69	.009
Temp x time x sample	8	2.757	5.14	.001
Residual	60	.536		
Calcium				
Temperature	2	.936	55.62	.001
Time	1	1.648	97.97	.001
Sample	4	287.319	17079.32	.001
Temperature x time	2	.622	36.95	.001
Temperature x sample	8	.530	31.49	.001
Time x sample	4	.547	32.49	.001
Temp x time x sample	8	.333	19.83	.001
Residual	59	.017		
Potassium				
Temperature	2	1.283	241.59	.001
Time	1	1.731	325.80	.001
Sample	4	4.712	886.99	.001
Temperature x time	2	.217	40.76	.001
Temperature x sample	8	.142	26.81	.001
Time x sample	4	.044	8.24	.001
Temp x time x sample	8	.014	2.60	.017
Residual	55	5.3×10^{-3}		
Zinc				
Temperature	2	17.041	.31	.739
Time	1	567.533	10.16	.003
Sample	4	23528.844	4210.54	.001
Temperature x time	2	502.839	9.00	.001
Temperature x sample	8	57.024	1.02	.434
Time x sample	4	166.516	2.98	.023
Temp x sample x time	8	89.643	1.60	.148
Residual	49	55.881		

Table B1. (Continued)

Source of variation	DF	Mean square	F	Prov>F
Copper				
Temperature	2	61.429	9.21	.001
Time	1	137.251	20.57	.001
Sample	4	2046.005	306.59	.001
Temperature x time	2	111.474	16.70	.001
Temperature x sample	8	28.616	4.29	.001
Time x sample	4	48.316	7.24	.001
Temp x time x sample	8	35.324	5.29	.001
Residual	50	6.673		

Duncan's multiple range test (P<.05)

	Ash	Ca	K	Zn	Cu
Temperature					
500	17.21^{a}	2.28^{b}	1.64^{a}	85.0^{a}	15.0^{b}
550	17.47^{a}	2.63^{a}	1.46^{b}	94.1^{a}	17.2^{a}
6.25	16.24^{b}	2.28^{b}	1.20^{c}	92.1^{a}	17.2^{a}
Time					
1:30	17.17^{a}	2.60^{a}	1.56^{a}	89.8^{a}	15.2^{b}
4:00	16.78^{b}	2.18^{b}	1.30^{b}	91.1^{a}	18.1^{a}
Sample					
Alfalfa hay	8.01^{d}	1.33^{b}	1.55^{c}	24.9^{c}	16.7^{c}
Corn stover	15.85^{b}	.61^{c}	.53^{e}	24.1^{c}	7.0^{c}
DP waste	42.61^{a}	9.86^{a}	1.32^{d}	298.1^{a}	31.7^{a}
Oat straw	11.62^{c}	.32^{d}	1.74^{b}	12.8^{d}	4.2^{e}
Soybean meal	6.78^{e}	.28^{d}	1.96^{a}	66.8^{b}	22.3^{b}

Table B2. Analysis of variance of ash, Ca, K, Zn, Cu, and Mg content of samples ashed at 4 different temperatures for 1.5 h

Source of variation	DF	Mean square	F	Prob>F
Ash				
Temperature	3	6.188	9.25	.001
Sample	4	2583.478	3862.7	.001
Temperature x sample	12	2.038	3.05	.004
Residual	40	.669		
Calcium				
Temperature	3	1.073	19.47	.001
Sample	4	231.969	4206.65	.001
Temperature x sample	12	.565	10.25	.001
Residual	40	.055		
Potassium				
Temperature	3	.784	286.84	.001
Sample	4	4.329	1583.76	.001
Temperature x sample	12	.048	17.54	.001
Residual	39	2.7×10^{-3}		
Zinc				
Temperature	3	131.735	5.43	.004
Sample	4	158972.762	6550.99	.001
Temperature x sample	12	66.670	2.75	.010
Residual	35	24.267		
Copper				
Temperature	3	30.064	22.73	.001
Sample	4	1132.949	856.50	.001
Temperature x sample	12	8.183	6.19	.001
Residual	38	1.323		
Magnesium				
Temperature	2	.001	9.7	.001
Sample	4	.248	3332.2	.001
Temperature x sample	8	.001	7.9	.001
Residual	30	7.4×10^{-5}		

Table B3. Analysis of variance of the IVDMD of 5 samples and the ash content of their residues determined by either the one-stage or the two-stage procedure followed by either normal or "exhaustive" washing of the residue, trial I

Source of variation	DF	Mean square	F	Prob>F
IVDMD				
Digestion phase (DP)	1	420.61	225.06	.001
Washing (W)	1	17.26	9.24	.004
Sample (S)	4	2488.59	1331.60	.001
DP x W	1	19.95	10.68	.002
DP x S	4	334.76	179.12	.001
W x S	4	50.09	26.80	.001
DP x W x S	4	24.58	13.15	.001
Residual	40	1.87		
Ash content of the residue				
Digestion phase (DP)	1	1135.09	1247.18	.001
Washing (W)	1	.17	.19	.666
Sample (S)	4	3329.42	3658.20	.001
DP x W	1	2.42	3.75	.068
DP x S	4	186.60	205.03	.001
W x S	4	2.25	2.47	.079
DP x W x S	4	9.38	10.31	.001
Residual	19	.91		

Duncan's multiple range test (P<.05)

	IVDMD	Ash
Digestion phase		
One-stage	60.99[b]	25.37[a]
Two-stage	66.28[a]	14.57[b]
Washing		
Normal	64.17[a]	19.90[a]
"Exhaustive"	63.10[b]	19.76[a]
Sample		
Alfalfa hay	64.34[b]	6.61[d]
Corn stover	56.76[c]	22.65[b]
Dry poultry waste	52.51[d]	54.01[a]
Oat straw	56.34[c]	9.48[c]
Soybean meal	88.23[a]	4.49[e]

Table B4. Analysis of variance of solubilization of DM and ash of 5 samples by 4 different enzymes

Source of variation	DF	Mean square	F	Prob>F
DM solubilized				
Treatment	5	256.16	1485.83	.001
Sample	4	2897.62	16807.03	.001
Treatment x sample	20	165.83	961.82	.001
Residual	30	.17		
Contrast:				
Buffers vs enzymes	1	1244.72	7219.76	.001
Buffers (pH 7.0 vs pH 4.8)	1	3.74	21.70	.001
Protease vs amylase	1	.24	1.40	.245
Cellulase A. niger vs cellulase T. viride	1	31.10	180.39	.001
pH 4.8 enzymes vs pH 7.0 enzymes	1	1.02	5.90	.021
Ash solubilized				
Treatment	5	672.72		.001
Sample	4	7001.82		.001
Treatment x sample	20	175.62		.001
Residual	30	.12		
Contrast:				
Buffers vs enzymes	1	206.96	1723.07	.001
Buffers (pH 7.0 vs pH 4.8)	1	1513.10	12597.93	.001
Protease vs amylase	1	63.58	529.37	.001
A. niger cellulase vs T. viride cellulase	1	1.55	12.92	.001
pH 4.8 enzymes vs pH 7.0 enzymes	1	1578.42	13141.60	.001

Table B4. (Continued)

Source of variation	DF	Mean square	F	Prob>F
Duncan's multiple range test (P<.05)				
		DM solubilized		Ash solubilized
Treatment				
1 = pH 7.0 buffer		28.18^{d}		43.48^{f}
2 = Protease WT		37.68^{b}		48.05^{e}
3 = Amylase WT		37.46^{b}		51.62^{d}
4 = pH 4.8 buffer		27.31^{e}		60.87^{c}
5 = Cellulase WT		36.00^{c}		62.67^{a}
6 = T. viride cellulase		38.50^{a}		62.12^{b}
Sample				
Alfalfa hay		43.68^{b}		74.24^{b}
Corn stover		16.89^{e}		31.79^{e}
Dry poultry waste		32.99^{c}		32.16^{d}
Oat straw		22.34^{d}		51.02^{c}
Soybean meal		55.04^{a}		84.80^{a}

Table B5. Analysis of variance of the effect of 7 pretreatments on the DM solubilized by either cellulase WT or buffer alone at 40 C for 24 h and the ash content of the residue of extraction of alfalfay hay and corn stover

Source of variation	DF	Mean square	F	Prob>F
Dry matter solubilized				
Pretreatment	6	6.565	73.68	.001
Enzyme	1	319.256	3582.91	.001
Enzyme x pretreatment	6	1.159	13.01	.001
Sample	1	6938.219	77865.35	.001
Sample x pretreatment	6	1.716	19.25	.001
Sample x enzyme	1	266.702	2993.10	.001
Sample x pretreatment x enzyme	6	3.738	41.95	.001
Residual	28	.089		
Ash content of residue				
Pretreatment	6	1.307	16.16	.001
Enzyme	1	4.318	53.40	.001
Enzyme x pretreatment	6	1.403	17.35	.001
Sample	1	1659.963	20530.03	.001
Sample x pretreatment	6	2.272	28.10	.001
Sample x enzyme	1	24.831	307.11	.001
Sample x pretreatment x enzyme	6	1.516	18.74	.001
Residual	28			

Duncan's multiple range test (P<.05)

	DM solubilized	Ash in the residue
Pretreatment		
None	25.37^{d}	8.10^{ab}
H_2O	26.78^{c}	8.32^{a}
pH 4.8 buffer	26.94^{c}	7.88^{bc}
.001 N HCl	27.07^{c}	7.30^{ef}
.01 N HCl	27.61^{b}	7.65^{cd}
.1 N HCl	27.59^{b}	7.56^{de}
2N HCl	28.24^{a}	7.22^{f}
Enzymes		
Buffer only	24.70^{b}	7.44^{b}
Cellulase WT	29.47^{a}	8.00^{a}
Sample		
Alfalfa hay	38.22^{a}	2.27^{b}
Corn stover	15.96^{b}	13.16^{a}

Table B6. Analysis of variance of the effect of HCl and pepsin-HCl pretreatments on the solubilization of DM, CP, and ash of alfalfa hay and corn stover by increasing levels of T. viride cellulase enzyme

Source of variation	DF	Mean square	F	Prob>F
DM solubilized				
Trial	1	28.04	28.19	.001
Sample	1	11929.01	11992.78	.001
Pretreatment	1	181.24	182.21	.001
Sample x pretreatment	1	154.94	155.77	.001
Enzyme	3	1937.28	1947.63	.001
Sample x enzyme	3	7.64	7.68	.001
Pretreatment x enzyme	3	15.27	15.35	.001
Sample x pretreatment x enzyme	3	16.72	16.81	.001
Residual	47	.99		
CP solubilized				
Sample	1	2381.54	1598.14	.001
Pretreatment	1	2504.90	1680.93	.001
Sample x pretreatment	1	377.71	253.47	.001
Enzyme	3	402.66	270.20	.001
Sample x enzyme	3	.88	.59	.630
Pretreatment x enzyme	3	153.38	102.93	.001
Sample x pretreatment x enzyme	3	15.48	10.39	.001
Residual	16	1.49		
Ash solubilized				
Sample	1	17188.90	35484.24	.001
Pretreatment	1	.02	.04	.851
Sample x pretreatment	1	.40	.83	.375
Enzyme	3	64.48	133.11	.001
Sample x enzyme	3	18.79	38.79	.001
Pretreatment x enzyme	3	0.88	1.82	.184
Sample x pretreatment x enzyme	3	1.45	3.00	.062
Residual	16	.48		

Table B6. (Continued)

Duncan's multiple range test (P<.05)	DM solubilized	CP solubilized	Ash solubilized
Sample			
Alfalfa hay	62.17[a]	70.39[a]	95.19[a]
Corn stover	34.86[b]	53.14[b]	48.83[b]
Pretreatment			
HCl	48.83[b]	52.92[b]	72.03[a]
Pepsin-HCl	50.20[a]	70.61[a]	71.99[a]
Enzyme			
Buffer only	32.35[c]	51.32[c]	68.02[c]
6.25	55.38[a]	63.32[b]	74.71[a]
12.50	55.58[a]	66.01[a]	72.45[b]
25.00	50.76[b]	66.40[a]	72.86[b]

Table B7. Analysis of variance of the disappearance of DM, OM, CP, ash, and P of alfalfa hay and corn stover samples in nylon bags suspended in the rumen for 12, 24, 48, and 72 h, followed by an incubation with pepsin-HCl for 24 h

Source of variation	DF	Mean square	F	Prob>F
Disappearance of DM				
Sample	1	2770.18	2006.37	.001
Digestion time	3	1314.47	952.04	.001
Sample x digestion time	3	52.85	38.28	.001
Residual	8	1.38		
Disappearance of OM				
Sample	1	2215.82	1489.42	.001
Digestion time	3	1429.03	960.56	.001
Sample x digestion time	3	56.47	37.96	.001
Residual	8	1.49		
Disappearance of CP				
Sample	1	20416.84	11745.42	.001
Digestion time	3	5373.62	3091.34	.001
Sample x digestion time	3	1096.75	630.94	.001
Residual	8	1.74		
Disappearance of ash				
Sample	1	14042.84	27855.53	.001
Digestion time	3	543.24	1077.58	.001
Sample x digestion time	3	77.87	154.46	.001
Residual	8	.50		
Disappearance of P				
Sample	1	44930.22	50873.88	.001
Digestion time	3	5624.37	6368.40	.001
Sample x digestion time	3	2920.61	3306.97	.001
Residual	8	.88		

Table B8. GENSTAT regression analysis of the disappearance of DM (DDM), OM (DOM), CP (DCP), ash (DAsh), and P(DP) of 2 samples in nylon bags suspended in the rumen for 12, 24, 48, and 72 h, followed by a 24 h incubation in pepsin-HCl

	Coefficients					
	Intercept	Linear	Quadratic	R^2	Prob>F	RSD
DDM						
Linear				.974	.01	7.43
Alfalfa hay	32.12	.56				
Corn stover	.14	.70				
Quadratic				.996	.01	2.89
Alfalfa hay	7.54	2.23	-.020			
Corn stover	-13.70	1.65	-.011			
DOM						
Linear				.973	.01	7.35
Alfalfa hay	28.41	.58				
Corn stover	-1.71	.75				
Quadratic				.996	.01	2.70
Alfalfa hay	3.62	2.27	-.020			
Corn stover	-14.93	1.65	-.011			
DCP						
Linear				.889	.01	19.05
Alfalfa hay	28.60	.89				
Corn stover	-72.70	1.66				
Quadratic				.934	.01	14.71
Alfalfa hay	-18.41	4.09	-.038			
Corn stover	-101.02	3.58	-.023			
DAsh						
Linear				.984	.01	8.35
Alfalfa hay	75.44	.32				
Corn stover	14.16	.37				
Quadratic				.995	.01	4.77
Alfalfa hay	53.22	1.83	-.018			
Corn stover	-4.37	1.63	-.015			

Table B8. (Continued)

	Coefficients					
	Intercept	Linear	Quadratic	R^2	Prob>F	RSD
DP						
Linear				.840	.01	28.32
Alfalfa hay	60.16	.44				
Corn stover	-102.42	1.89				
Quadratic				.820	.01	30.00
Alfalfa hay	50.48	1.10	-.008			
Corn stover	-130.07	3.87	-.022			

Table B9. Analysis of the disappearance of DM, OM, CP, ash, and P of alfalfa hay and corn stover samples in nylon bags suspended in the rumen for 24, 48 and 72 h, followed by incubation in pepsin-HCl

Source of variation	DF	Mean square	F	Prob>F
Disappearance of DM (DDM)				
Trial	2	135.35	15.73	.001
Sample	1	7532.97	875.63	.001
Digestion time	2	951.00	110.55	.001
Sample x digestion time	2	157.51	18.31	.001
Residual	37	8.60		
Disappearance of OM (DOM)				
Trial	2	139.17	15.41	.001
Sample	1	6195.53	686.13	.001
Digestion time	2	1117.30	123.74	.001
Sample x digestion time	2	181.58	20.11	.001
Residual	37	9.03		
Disappearance of CP (DCP)				
Trial	2	454.72	13.15	.001
Sample	1	22023.58	636.66	.001
Digestion time	2	367.23	10.62	.001
Sample x digestion time	2	337.22	9.75	.001
Residual	37	34.59		
Disappearance of ash (DAsh)				
Trial	2	123.89	13.44	.001
Sample	1	32404.24	3514.28	.001
Digestion time	2	84.15	9.13	.001
Sample x digestion time	2	102.86	11.15	.001
Residual	37	9.22		
Disappearance of P (DP)				
Trial	2	1506.81	16.21	.001
Sample	1	37089.96	398.89	.001
Digestion time	2	392.07	4.22	.022
Sample x digestion time	2	809.73	8.71	.001
Residual	37	92.98		

Table B9. (Continued)

Duncan's multiple range test (P<.05)	DDM	DOM	DCP	DAsh	DP
Sample					
Alfalfa hay	64.78[a]	62.05[a]	88.94[a]	95.14[a]	92.36[a]
Corn stover	36.72[b]	36.31[b]	43.23[b]	39.46[b]	36.19[b]
Digestion time					
24 h	43.23[c]	41.05[c]	65.93[b]	65.04[c]	69.22[a]
48 h	54.08[b]	52.60[b]	63.31[b]	69.97[b]	62.03[b]
72 h	61.38[a]	60.30[a]	75.50[a]	74.32[a]	66.70[ab]

Table B10. Regression coefficients of the linear regression analysis of the disappearance of DM, OM, CP, and ash according to 2 different laboratory techniques

	X = solubility in pepsin-cellulase (%) coefficient						
	Intercept	Linear	R^2	DF	RSD[a]	Prob>F	CV
Y = disappearance from nylon bag-pepsin (%)							
Dry matter	17.51	.79	.77	13	5.78	.0001	11.31
Organic matter	17.25	.82	.77	13	5.98	.0001	11.89
Crude protein	-46.74	1.61	.91	13	5.58	.0001	8.61
Ash	4.39	.93	.84	13	6.95	.0001	10.31

[a]RSD = residual standard deviation.

Table B11. Analysis of variance of the disappearance of DM (DDM), OM (DOM), CP (DCP), and ash (DAsh) of 15 samples determined by the nylon bag-pepsin and the pepsin-cellulase techniques

Source of variation	DF	Mean square	F	Prob>F
DDM in nylon bag-pepsin				
Sample	14	368.99	95.79	.001
Residual	24	3.85		
DDM in pepsin-cellulase				
Sample	14	633.10	155.81	.001
Residual	44	5.06		
DOM in nylon bag-pepsin				
Sample	14	393.57	116.19	.001
Residual	24	3.39		
DOM in pepsin-cellulase				
Sample	14	621.64	145.13	.001
Residual	44	4.28		
DCP in nylon bag-pepsin				
Sample	14	797.42	29.21	.001
Residual	24	27.30		
DCP in pepsin-cellulase				
Sample	14	429.40	81.42	.001
Residual	44	5.27		
DAsh in nylon bag-pepsin				
Sample	14	758.68	55.83	.001
Residual	24	13.59		
DAsh in pepsin-cellulase				
Sample	14	1074.02	62.56	.001
Residual	44	17.17		

Table B12. Analysis of variance of the effect of a 2nd stage of digestion with pepsin-HCl and time of digestion in the rumen on the disappearance of DM (DDM), OM (DOM), CP (DCP), ash (DAsh), Ca (DCa), P (DP), Mg (DMg), K (DK), Zn (DZn), Mn (DMn), and Cu (DCu) in nylon bags

Source of variation	DF	Mean square	F	Prob>F
DDM				
Sample	1	3995.92	1713.75	.001
Digestion time (DT)	2	1095.45	469.81	.001
Sample x DT	2	211.92	90.89	.001
Pepsin	1	12.09	5.18	.032
Sample x pepsin	1	13.47	5.78	.024
DT x pepsin	2	16.95	7.27	.003
Sample x DT x pepsin	2	7.50	3.22	.058
Residual	24	2.33		
DOM				
Sample	1	3199.41	1307.31	.001
DT	2	1310.24	535.38	.001
Pepsin	1	3.19	1.30	.265
Sample x DT	2	248.41	101.50	.001
Sample x pepsin	1	9.92	4.05	.055
DT x pepsin	2	17.14	7.00	.040
Sample x DT x pepsin	2	7.71	3.15	.061
Residual	24	2.45		
DCP				
Sample	1	19701.40	6382.77	.001
DT	2	194.96	63.16	.001
Sample x DT	2	223.17	73.30	.001
Pepsin	1	2245.65	727.54	.001
Sample x pepsin	1	388.16	125.75	.001
DT x pepsin	2	109.50	35.48	.001
Sample x DT x pepsin	2	24.84	8.05	.002
Residual	24	3.09		
DAsh				
Sample	1	18897.08	12225.06	.001
DT	2	52.60	34.03	.001
Sample x DT	2	108.11	69.94	.001
Pepsin	1	406.29	262.84	.001
Sample x pepsin	1	131.33	84.96	.001
DT x pepsin	2	16.19	10.47	.001
Sample x DT x pepsin	2	6.02	3.89	.034
Residual	24			

Table B12. (Continued)

Source of variation	DF	Mean square	F	Prob>F
DCa				
Sample	1	3.39	5.80	.024
DT	2	36.10	61.76	.001
Sample x DT	2	11.60	19.84	.001
Pepsin	1	5378.51	9202.12	.001
Sample x pepsin	1	3.33	5.70	.025
DT x pepsin	2	42.92	73.43	.001
Sample x DT x pepsin	2	7.56	12.93	.001
Residual	24	.58		
DP				
Sample	1	35909.62	8561.17	.001
DT	2	1189.29	283.54	.001
Sample x DT	2	1420.63	338.69	.001
Pepsin	1	2824.39	673.36	.001
Sample x pepsin	1	1672.40	398.71	.001
DT x pepsin	2	57.99	13.83	.001
Sample x DT x pepsin	2	71.59	17.07	.001
Residual	24	4.19		
DMg				
DT	2	29.98	158.01	.001
Pepsin	1	478.44	2521.33	.001
DT x pepsin	2	29.20	153.89	.001
Residual	12	.19		
DK				
Sample	1	120.49	5402.34	.001
DT	2	3.24	145.39	.001
Sample x DT	2	2.38	106.61	.001
Pepsin	1	95.97	4303.26	.001
Sample x pepsin	1	67.57	3029.60	.001
DT x pepsin	2	1.97	88.39	.001
Sample x DT x pepsin	2	1.40	62.97	.001
Residual	24	.02		
DZn				
Sample	1	74478.05	1775.46	.001
DT	2	47623.29	1135.28	.001
Sample x DT	2	7200.57	171.65	.001
Pepsin	1	353731.53	8432.51	.001
Sample x pepsin	1	74478.05	1776.46	.001
DT x pepsin	2	47623.29	1135.28	.001
Sample x DT x pepsin	2	7200.57	171.65	.001
Residual	24	41.95		

Table B12. (Continued)

Source of variation	DF	Mean square	F	Prob>F
DMn				
Sample	1	471.40	56.79	.001
DT	2	1433.82	172.74	.001
Sample x DT	2	646.77	77.92	.001
Pepsin	1	55657.46	6705.33	.001
Sample x pepsin	1	972.50	117.16	.001
DT x pepsin	2	1288.14	155.19	.001
Sample x DT x pepsin	2	748.32	90.25	.001
Residual	24	8.20		
DCu				
Sample	1	3173.44	3015.68	.001
DT	2	169.20	160.79	.001
Sample x DT	2	6.48	6.16	.007
Pepsin	1	1393.78	1324.49	.001
Sample x pepsin	1	30.80	29.27	.001
DT x pepsin	2	149.81	142.36	.001
Sample x DT x pepsin	2	95.12	90.39	.001
Residual	24	1.05		

Duncan's multiple range test (P<.05)

	DDM	DOM	DCP	DAsh	DCa	DP
Sample						
Alfalfa hay	64.58^{a}	62.32^{a}	85.26^{a}	89.68^{a}	86.41^{b}	92.35^{a}
Corn stover	43.51^{b}	43.46^{b}	38.48^{b}	43.85^{b}	87.02^{a}	29.19^{b}
Digestion time						
24 h	43.32^{c}	41.20^{c}	59.98^{b}	64.47^{c}	84.71^{b}	71.88^{a}
48 h	57.18^{b}	56.16^{b}	59.14^{b}	68.56^{a}	87.75^{a}	57.76^{b}
72 h	61.63^{a}	61.32^{a}	66.50^{a}	67.27^{b}	87.68^{a}	52.67^{c}
Pepsin						
With	54.62^{a}	53.19^{a}	69.77^{a}	70.12^{a}	98.94^{a}	69.63^{a}
Without	53.46^{b}	52.59^{a}	53.97^{b}	63.41^{b}	74.49^{b}	51.91^{b}

Table B12. (Continued)

Duncan's multiple range test (P<.05)	DMg	DK	DZn	DMn	DCu
Sample					
Alfalfa hay	94.72	99.69^{a}	46.36^{a}	55.48^{b}	82.71^{a}
Corn stover	-	96.03^{b}	-44.61^{b}	62.72^{a}	63.94^{b}
Digestion time					
24 h	94.14^{b}	97.28^{c}	-71.87^{b}	46.52^{b}	69.02^{c}
48 h	95.96^{a}	98.00^{b}	37.64^{a}	66.32^{a}	75.92^{a}
72 h	96.07^{a}	98.29^{a}	36.85^{a}	64.46^{a}	75.04^{b}
Pepsin					
With	99.88^{a}	99.49^{a}	100.00^{a}	98.42^{a}	79.55^{a}
Without	89.57^{b}	96.22^{b}	-98.25^{b}	19.78^{b}	67.10^{b}

Table B13. Analysis of variance of the effect of a 2nd stage of digestion with pepsin-HCl on the disappearance of DM (DDM), OM(DOM), CP, (DCP), ash (DAsh), Ca (DCa), P (DP), Mg (DMg), K (DK), Zn (DZn), Mn (DMn), and Cu (DCu) in nylon bags for 48 h

Source of variation	DF	Mean square	F	Prob>F
DDM				
Trial	1	67.47	3.89	.077
Sample	1	1129.93	65.22	.001
Pepsin	1	5.61	.32	.582
Sample x pepsin	1	10.87	.63	.447
Residual	10			
DOM				
Trial	1	79.87	4.59	.058
Sample	1	1315.64	75.53	.001
Pepsin	1	10.69	.61	.452
Sample x pepsin	1	10.78	.62	.450
Residual	10	17.42		
DCP				
Trial	1	35.82	2.32	.159
Sample	1	3795.15	245.59	.001
Pepsin	1	526.28	34.06	.001
Sample x pepsin	1	84.55	5.47	.041
Residual	10	15.45		
DAsh				
Trial	1	.81	.03	.860
Sample	1	112.54	4.58	.058
Pepsin	1	118.29	4.81	.053
Sample x pepsin	1	.40	.02	.901
Residual	10	24.58		
DCa				
Trial	1	21.85	.70	.422
Sample	1	97.19	3.12	.106
Pepsin	1	14959.31	479.54	.001
Sample x pepsin	1	103.91	3.33	.098
Residual	10	31.20		

Table B13. (Continued)

Source of variation	DF	Mean square	F	Prob>F
DP				
Trial	1	39.13	7.70	.020
Sample	1	200.82	39.52	.001
Pepsin	1	391.40	77.03	.001
Sample x pepsin	1	15.46	3.04	.112
Residual	10	5.08		
DMg				
Trial	1	11.37	6.42	.030
Sample	1	88.36	49.91	.001
Pepsin	1	818.31	462.20	.001
Sample x pepsin	1	83.05	46.91	.001
Residual	10	1.77		
DK				
Trial	1	.112	3.31	.099
Sample	1	.008	.22	.646
Pepsin	1	3.718	110.39	.001
Sample x pepsin	1	.089	2.63	.136
Residual	10	.034		
DZn				
Trial	1	20100.63	7.67	.020
Sample	1	7205.69	2.75	.128
Pepsin	1	70354.77	26.85	.001
Sample x pepsin	1	7306.01	2.79	.126
Residual	10	2619.92		
DMn				
Trial	1	17.78	6.01	.034
Sample	1	1.50	.51	.492
Pepsin	1	710.92	240.26	.001
Sample x pepsin	1	1.50	.51	.492
Residual	10	2.96		
DCu				
Trial	1	1800.13	12.34	.006
Sample	1	9918.39	68.01	.001
Pepsin	1	3878.93	26.60	.001
Sample x pepsin	1	1970.11	13.51	.004
Residual	10	145.83		

Table B13. (Continued)

Duncan's multiple range test (P<.05)

	DDM	DOM	DCP	DAsh	DCa
Sample					
Corn silage	48.88[a]	48.66[a]	71.35[a]	53.58[a]	70.55[a]
Elephant grass silage	31.85[b]	30.29[b]	38.45[b]	47.58[b]	70.52[a]
Pepsin					
With	40.01[a]	38.91[a]	60.52[a]	53.19[a]	99.91[a]
Without	39.56[a]	38.81[a]	46.12[b]	47.17[b]	36.96[b]

	DP	DMg	DK	DZn	DMn	DCu
Sample						
Corn silage	87.20[a]	91.21[b]	99.45[a]	45.47[a]	94.46[a]	35.32[a]
Grass silage	78.98[b]	94.84[a]	99.33[a]	-.28[a]	92.72[a]	-15.78[b]
Pepsin						
With	87.84[a]	99.85[a]	99.86[a]	91.15[a]	100.00[a]	26.49[a]
Without	77.07[b]	85.48[b]	98.85[b]	-59.01[b]	86.14[b]	-12.99[b]

Table B14. Analysis of variance of the % loss of particulate DM and the % losses of water-soluble DM, OM, CP, ash, Ca, P, and Mg from nylon bags containing alfalfa hay and corn stover samples, during extraction with water.

Source of variation	DF	Mean square	F	Prob>F
		Trials 1, 2 and 3		
Loss of particulate DM				
Trial	2	52.80	41.00	.001
Sample	1	.06	.05	.831
Residual	10	1.29		
Water-soluble DM				
Trial	2	75.71	38.89	.001
Sample	1	842.89	432.95	.001
Residual	10	1.95		
Water-soluble OM				
Trial	2	85.59	48.80	.001
Sample	1	908.04	517.75	.001
Residual	10	1.75		
Water-soluble CP				
Trial	2	237.70	9.10	.006
Sample	1	203.53	7.79	.019
Residual	10	26.11		
Water-soluble ash				
Trial	2	108.98	33.56	.001
Sample	1	2400.18	739.05	.001
Residual	10	3.25		
Water-soluble Ca				
Trial	2	377.74	62.85	.001
Sample	1	91.85	15.28	.003
Residual	10	6.01		
Water-soluble P				
Trial	2	40.47	11.35	.003
Sample	1	450.54	126.41	.001
Residual	10	3.56		
Water-soluble Mg				
Trial	2	908.90	272.87	.001
Sample	1	125.58	37.70	.001
Residual	10	3.33		

Table B14. (Continued)

Source of variation	DF	Mean square	F	Prob>F
	Trial 4			
Loss of particulate DM				
Block	1	9.68	171.6	.0001
Sample	14	17.80	315.6	.0001
Residual	14			
Water-soluble DM				
Block	1	48.95	42.4	.0001
Sample	14	105.98	91.7	.0001
Residual	14	1.16		
Water-soluble CP				
Block	1	32.80	30.1	.0001
Sample	14	124.81	114.5	.0001
Residual	14	1.09		
Water-soluble ash				
Block	1	16.68	24.0	.0002
Sample	14	494.72	712.7	.0001
Residual	14	.69		
Water-soluble Ca				
Block	1	23.43	36.8	.0001
Sample	14	454.99	714.4	.0001
Residual	14	.64		
Water-soluble P				
Block	1	3.69	21.5	.0004
Sample	14	162.44	948.4	.0001
Residual	14	.17		
Water-soluble Mg				
Block	1	7.35	30.3	.0001
Sample	14	759.26	3127.7	.0001
Residual	14	.24		
Water-soluble K				
Block	1	.033	2.4	.14
Sample	14	22.33	1639.6	.0001
Residual	14	.014		
Water-soluble Mn				
Block	1	26.10	26.2	.0002
Sample	14	697.27	700.9	.0001
Residual	14	.99		

Table B14. (Continued)

Source of variation	DF	Mean square	F	Prob>F
	Trial 5			
Loss of particulate DM				
Sample	2	84.74	291.3	.0001
Residual	9	.29		
Water-soluble DM				
Sample	2	858.28	3515.0	.0001
Residual	9	.24		
Water-soluble CP				
Sample	2	570.07	52.1	.0001
Residual	9	10.94		
Water-soluble ash				
Sample	2	540.39	179.8	.0001
Residual	9	3.01		
Water-soluble Ca				
Sample	2	1821.65	64.3	.0001
Residual	9	28.31		
Water-soluble P				
Sample	2	60.93	51.5	.0001
Residual	9	1.18		
Water-soluble Mg				
Sample	2	5033.61	4540.0	.0001
Residual	9	1.11		
Water-soluble Zn				
Sample	2	335.11	8.2	.009
Residual	9	40.65		
	Trial 6			
Loss of particulate DM				
Sample size	2	.15	1.0	.41
Residual	6	.14		

Table B14. (Continued)

Duncan's multiple range test (P<.05)

	Loss DM	WSDM	WSOM	WSCP	WSA	WSCa	WSP	WSMg
Trial								
1	8.02[a]	22.72[b]	18.33[b]	42.44[a]	63.08[a]	52.42[a]	80.38[b]	83.92[a]
2	7.47[a]	26.16[a]	22.57[a]	36.57[a]	59.32[b]	52.18[a]	83.68[a]	81.82[a]
3	2.21[b]	18.34[c]	14.18[c]	28.62[b]	53.77[c]	37.46[b]	77.88[b]	59.90[b]
Sample[1]								
AH	5.31[a]	29.58[a]	25.81[a]	31.02[b]	71.11[a]	43.38[b]	85.92[a]	76.02[a]
CS	5.44[a]	14.06[b]	9.71[b]	38.65[a]	44.92[b]	48.50[a]	74.58[b]	70.04[b]

[1]AH = alfalfa hay and CS = corn stover.

Table B15. Regression analysis of the disappearance of DM (DDM), CP (DCP), ash (DAsh), Ca (DCa), P (DP), Mg (DMg), and Mn (DMn) of feed samples contained in nylon bags digested in the rumen of fistulated animals, trials 1 and 2

	Coefficients					
	Intercept	Linear	Quadratic	R^2	Prob>F	RSD
			Trial 1			
Alfalfa, 2nd cut						
DDM	46.96	.59	-.0038	.75	.0001	2.62
DCP	59.75	.80	-.0055	.92	.0001	1.50
DAsh	84.22	.17	-.0010	.75	.0001	.84
DCa	56.67	.39	-.0021	.73	.0001	2.51
DP	86.85	.18	-.0013	.72	.0001	.61
DMg	76.81	.44	-.0033	.81	.0001	1.12
DMn	0.0	.54	0.0	.84	.0001	5.32
Tall fescue						
DDm	30.40	.88	-.0048	.80	.0001	4.54
DCP	43.20	1.12	-.0074	.89	.0001	2.74
DAsh	71.12	0.0	0.0	.17	.07	3.56
DCa	54.06	.70	-.0048	.70	.0001	2.90
DP	85.70	.19	-.0012	.65	.0001	1.01
DMg	89.87	.11	-.0008	.33	.004	.97
DMn	72.50	.24	0.0	.58	.0001	2.22
Soybean pods						
DDM	31.51	.79	-.0043	.83	.0001	3.64
DCP	36.49	1.18	-.0076	.92	.0001	2.66
DAsh	82.38	.28	-.0017	.88	.0001	.84
DCa	51.80	.87	-.0054	.92	.0001	2.15
DP	68.48	.76	-.0065	.88	.0001	1.44
DMg	89.18	.25	-.0017	.93	.0001	.47
DMn	0.0	2.12	-.0140	.91	.0001	4.80
Soybean stalks						
DDM	8.95	.63	-.0042	.85	.0001	2.06
DCP	32.43	0.0	0.0	.91	.0001	1.63
DAsh	67.64	0.0	.0016	.95	.0001	.79
DCa	47.61	1.10	-.0081	.99	.0001	.63
DP	65.85	.24	-.0016	.85	.0001	.77
DMg	82.44	.39	-.0038	.96	.0001	.27
DMn	-42.82	2.55	-.0210	.97	.0001	2.21

Table B15. (Continued)

	Coefficients					
	Intercept	Linear	Quadratic	R^2	Prob>F	RSD
Soybean stover						
DDM	18.90	.50	-.0030	.77	.0001	2.46
DCP	38.78	.64	-.0053	.68	.0001	2.22
DAsh	57.30	.32	-.0016	.87	.0001	1.42
DCa	60.30	.47	-.0029	.72	.0001	2.31
DP	64.62	0.0	0.0	.47	.0001	2.92
DMg	89.82	.07	-.0004	.61	.0001	.64
DMn	8.31	1.27	-.0091	.80	.0001	3.71
			Trial 2			
Alfalfa hay						
DDM	7.80	6.22	-.1512	.91	.0001	4.13
DCP	0.0	8.36	-.2065	.94	.0001	4.08
DAsh	53.79	2.96	-.0792	.76	.0001	2.73
DCa	0.0	7.28	-.1849	.84	.0001	5.91
DP	68.60	0.0	0.0	.18	.007	5.60
DMg	67.68	2.88	-.0751	.84	.0001	2.24
DZn	0.0	5.89	-.1481	.72	.0001	7.13
Corn stover silage						
DDM	-6.29	2.43	-.0318	.93	.0001	3.12
DCP	36.93	-3.26	.0866	.24	.002	9.71
DAsh	40.33	0.0	0.0	.13	.04	4.89
DCa	-14.52	4.11	-.0815	.76	.0001	7.02
DP	72.35	-4.14	0.0	.50	.0001	14.34
DMg	70.81	.84	-.0172	.56	.0001	2.17
DZn	21.70	0.0	0.0	.31	.0001	13.58
Corn cobs						
DDM	-11.48	2.91	-.0342	.93	.0001	4.02
DCP	100.20	-22.15	.5082	.82	.0001	24.77
DAsh	42.34	0.0	0.0	.13	.03	13.55
DCa	-625.55	0.0	0.0	.01	.71	73.24
DP	188.95	-35.07	.6809	.87	.0001	41.95
DMg	22.87	0.0	0.0	.19	.006	7.11
DZn	77.94	-2.71	0.0	.57	.0001	8.83

Table B16. Analysis of variance of the intake of DM and mineral elements, and the excretion of mineral elements of sheep fed corn silage and elephant grass silage supplemented with four levels of mineral salts held in metabolism cages

Source of variation	DF	Mean square	F	Prob>F
DM intake from silage, kg/day				
Block	2	.035	2.53	.118
Silage	1	.150	10.78	.006
Mineral mixture	3	.002	.11	.950
Silage x mineral mixture	3	.007	.51	.682
Residual	13	.014		
Total DM intake, $g/kg^{0.75}$/day				
Block	2	4.95	.12	.888
Silage	1	331.36	8.02	.014
Mineral mixture	3	6.36	.15	.925
Silage x mineral mixture	3	25.21	.61	.620
Residual	13	41.33		
CA intake from silage, g/day				
Block	2	.084	.95	.411
Silage	1	11.886	133.44	.001
Mineral mixture	3	.044	.49	.696
Silage x mineral mixture	3	.099	1.11	.378
Residual	14	.089		
P intake from silage, g/day				
Block	2	.051	1.17	.339
Silage'	1	.230	5.25	.038
Mineral mixture	3	.005	.12	.945
Silage x mineral mixture	3	.044	1.01	.420
Residual	14	.044		
Mg intake from silage				
Block	2	.072	1.11	.356
Silage	1	.267	4.11	.062
Mineral mixture	3	.015	.22	.878
Silage x mineral mixture	3	.071	1.10	.381
Residual	14	.065		
Zn intake from silage, mg/day				
Block	2	7.271	1.23	.322
Silage	1	327.082	55.39	.001
Mineral mixture	3	.197	.03	.991
Silage x mineral mixture	3	4.686	.79	.518
Residual	14	5.905		

Table B16. (Continued)

Source of variation	DF	Mean square	F	Prob>F
Mn intake from silage, mg/day				
Block	2	299.87	.85	.449
Silage	1	66834.26	188.88	.001
Mineral mixture	3	235.91	.67	.586
Silage x mineral mixture	3	427.13	1.21	.343
Residual	14	353.85		
Cu intake from silage, mg/day				
Block	2	.22	1.17	.339
Silage	1	21.64	113.51	.001
Mineral mixture	3	.01	.05	.984
Silage x mineral mixture	3	.16	.85	.483
Residual	14	.19		
Total Ca excretion in feces, g/day				
Block	2	.50	3.66	.053
Silage	1	15.42	112.87	.001
Mineral mixture	3	32.18	235.49	.001
Silage x mineral mixture	3	.16	1.17	.356
Residual	14	.14		
Total P excretion in feces, g/day				
Block	2	.12	.90	.430
Silage	1	.47	3.41	.086
Mineral mixture	3	6.42	46.58	.001
Silage x mineral	3	.45	3.26	.054
Residual	14	.14		
Total Mg excretion in feces, g/day				
Block	2	.013	.67	.528
Silage	1	1.782	89.52	.001
Mineral mixture	3	.072	3.63	.040
Silage x mineral mixture	3	.090	4.55	.020
Residual	14			

Table B17. Analysis of variance of the apparent digestibility and nitrogen balance of corn silage and elephant grass silage supplemented with four levels of mineral elements to sheep in metabolism cages

Source of variation	DF	Mean square	F	Prob>F
Apparent digestibility of DM				
Block	2	7.55	.65	.539
Silage	1	151.31	27.67	.001
Mineral mixture	3	2.54	.47	.712
Silage x mineral mixture	3	7.71	1.41	.284
Residual	13	5.47		
Apparent digestibility of OM				
Block	2	3.32	.59	.566
Silage	1	115.34	20.64	.001
Mineral mixture	3	1.68	.30	.824
Silage x mineral mixture	3	6.89	1.23	.337
Residual	13	5.59		
Apparent digestibility of the gross energy				
Block	2	2.27	.20	.823
Silage	1	89.33	6.50	.024
Mineral mixture	3	1.64	.12	.947
Silage x mineral mixture	3	8.09	.59	.633
Residual	13	13.74		
Apparent digestibility of the CP				
Block	2	5.76	.63	.550
Silage	1	17.12	1.86	.196
Mineral mixture	3	6.19	.67	.584
Silage x mineral mixture	3	17.65	1.92	.176
Residual	13	9.20		
Apparent absorption of the ash				
Block	2	15.94	1.56	.246
Silage	1	.46	.05	.834
Mineral mixture	3	67.24	6.60	.006
Silage x mineral mixture	3	36.16	3.55	.045
Residual	13	10.19		
Nitrogen balance				
Block	2	.002	1.04	.381
Silage	1	.112	60.80	.001
Mineral mixture	3	.002	.86	.488
Silage x mineral mixture	3	.002	.98	.431
Residual	13	.002		

Table B17. (Continued)

Source of variation	DF	Mean square	F	Prob>F
Apparent absorption of Ca				
Block	2	102.04	1.50	.257
Silage	1	169.76	2.50	.136
Mineral mixture	3	271.26	3.99	.030
Silage x mineral mixture	3	85.72	1.26	.326
Apparent absorption of P				
Block	2	56.14	.87	.441
Silage	1	933.75	14.46	.002
Mineral mixture	3	60.50	.94	.449
Silage x mineral mixture	3	105.64	1.64	.226
Residual	14	64.57		
Apparent absorption of Mg				
Block	2	9.52	.26	.776
Silage	1	3154.48	85.79	.001
Mineral mixture	3	118.44	3.22	.055
Silage x mineral mixture	3	79.71	2.17	.137
Residual	14	36.77		
Apparent absorption of Zn				
Block	2	75.74	.30	.746
Silage	1	17417.79	68.87	.001
Mineral mixture	3	854.88	3.38	.049
Silage x mineral mixture	3	1295.51	5.12	.013
Residual	14	252.51		
Apparent absorption of Mn				
Block	2	8.17	.14	.870
Silage	1	4568.90	78.58	.001
Mineral mixture	3	543.88	9.35	.001
Silage x mineral mixture	3	22.43	.39	.765
Residual	14	58.14		
Apparent absorption of Cu				
Block	2	143.81	.46	.639
Silage	1	20073.37	64.65	.001
Mineral mixture	3	3529.67	11.37	.001
Silage x mineral mixture	3	3440.02	11.08	.001
Residual	14	310.51		

Table B18. Analysis of variance of the calculated true absorption of mineral elements in corn silage and elephant grass silage supplemented with four levels of mineral elements by sheep

Source of variation	DF	Mean square	F	Prob>F
Calculated true absorption of Ca				
Block	2	130.23	3.53	.058
Silage	1	2600.83	70.43	.001
Mineral mixture	3	774.24	20.97	.001
Silage x mineral mixture	3	241.44	6.54	.005
Residual	14	36.93		
Calculated true absorption of P				
Block	2	109.49	3.71	.059
Silage	1	965.24	32.70	.001
Mineral mixture	3	245.43	8.31	.004
Silage x mineral mixture	3	74.52	2.52	.111
Residual	11	29.52		
Calculated true absorption of Mg				
Block	2	12.49	.42	.663
Silage	1	3515.78	119.24	.001
Mineral mixture	3	143.36	4.83	.016
Silage x mineral mixture	3	103.25	3.50	.044
Residual	14	29.48		
Calculated true absorption of Zn				
Block	2	184.92	.83	.456
Silage	1	14084.59	63.60	.001
Mineral mixture	3	370.94	1.67	.221
Silage x mineral mixture	3	1309.95	5.91	.009
Residual	13	221.47		
Calculated true absorption of Cu				
Block	2	153.29	.49	.623
Silage	1	19598.45	62.56	.001
Mineral mixture	3	3067.71	9.79	.001
Silage x mineral mixture	3	3291.93	10.51	.001
Residual	14	313.26		